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RATE-CONTROLLED DELIVERY SYSTEMS IN DRUG AND HORMONE RESEARCH

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INTRODUCTION

The advent of implantable devices that give long-duration control over the rate of drug or hormone administration in laboratory animals has opened up both novel and previously impractical experimental methods, protocols, and models for delivering these substances. This review brings together these new methods, protocols, and models for examination from the general perspectives of experimental pharmacology, toxicology, and physiology. They have originated in various specialized fields that infrequently cross-communicate, yet they have a common theme in the controlled deployment of bioactive agents in experimental animals. The novelty in this theme stems both from recent technological advances in drug delivery systems and from the ingenious ways in which individual researchers have put them to use. The focus here is on uses of delivery systems rather than on their technical aspects, which have been well-reviewed elsewhere (1, 2). Some history of the development of these delivery systems is pertinent, however.

Only during the past two decades have practical means existed for multiday, rate-controlled administration of drugs or hormones to both experimental animals and man. Most of the technical advances in delivery systems for clinical use, including infusion pumps, have come since 1974. Among systems for research use, little progress was made with external pumps because of the intricacies of maintaining a leak- and kink-proof flow path from a stationary pump to an uncooperatively revolving animal. An early development was the Rose-Nelson osmotic pump (3)—too bulky for rodents but small enough to be worn externally by dogs—allowing the first multiday infusions of angiotensin

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II (4, 5). Another stage of miniaturization was needed to make implantable systems suitable for use in rats, mice, and other small laboratory animals.

The first such implants were demonstrated by Folkman & Long in 1964 (6), who showed that drug- or hormone-filled polysiloxane (SILASTIC®) capsules could provide extended-duration, rate-controlled administration of the contained substance by the process of solution diffusion (7). However, these capsules' uses are limited to substances, principally steroids, whose combination of high molar potency and solubility in polysiloxane gives them usefully high rates of release; peptides, as a rule, and many drugs lack the requisite solubility in polysiloxane, and most drugs lack the requisite molar potency.

The development of miniature implantable osmotic pumps in the mid-1970s (8) greatly expanded the range of drugs and hormones, including peptides, for which multiday constant or programmed-rate administration was possible in mice, rats, and larger animals. Osmotic pumps provide a convective stream of drug solution that can be directed through suitable catheter connections to sites in the animal remote from the pump itself, a feature that researchers have ingeniously exploited in many ways. Also in the mid-1970s Folkman & Langer reported a method of loading various agents, including peptides, in ethylene vinyl acetate copolymer pellets to achieve extended-duration release after the pellets are implanted subcutaneously or directly in various tissues, for example the cornea (9); these pellets have played an important role in studies on the biology of new vessel growth into tumors (10).

Although this review concerns drug delivery systems in animal experimentation, it is useful to note briefly recent developments in human pharmaceuticals based on drug delivery systems (11). A number of rate-controlled topical and systemic pharmaceuticals have been registered for human use. Topical products are pilocarpine in a seven-day ocular delivery system (12) and progesterone in a one-year uterine delivery system (13, 14); systemic products are oral forms of theophylline (15) and indomethacin (16, 17) and several transdermal products including three-day scopolamine (18) and one-day nitroglycerin (19, 20); a seven-day transdermal form of clonidine is in advanced clinical trials (21). Concomitantly, there has been a big increase in use of the infusion mode of intravenous drug therapy and the development of many kinds of infusion pumps and controllers for in-hospital use. Drugs that require rate-controlled intravenous (iv) infusion have begun to come into use: dopamine, dobutamine, nitroprusside, and alprostadil. An implantable pump is now in clinical use for chronic infusions (22).

Basic researchers have much more diverse needs for different agents and regimens than do clinicians. Consequently, delivery systems for basic research have been designed to facilitate use with a broad range of agents, minimizing agent-specific formulational issues. Delivery systems for basic research are in

effect empty devices that, with due regard for inherent limitations of size, material incompatibilities, and agent solubility, can readily be loaded to infuse whatever agent the researcher chooses. In contrast, delivery systems-based pharmaceuticals are integral units from which it is impossible to remove the drug and substitute another without destroying the system.

This review is organized along the lines of the important new methods, protocols, and models based on delivery system use. It draws on a literature that numbers over 700 publications spanning pharmacology, toxicology, physiology, endocrinology, neural science, environmental studies, experimental psychology, and zoology. It covers literature through mid-1983.

Two Illustrative Studies

Several general principles emerge from analyzing two studies of systemic drug action. Figures 1 and 2, from the work of Smits et al (23), show the hemodynamic responses of the spontaneously hypertensive (SH) rat to propranolol given by constant-rate, five-day infusion (Figure 1) or as a single injection

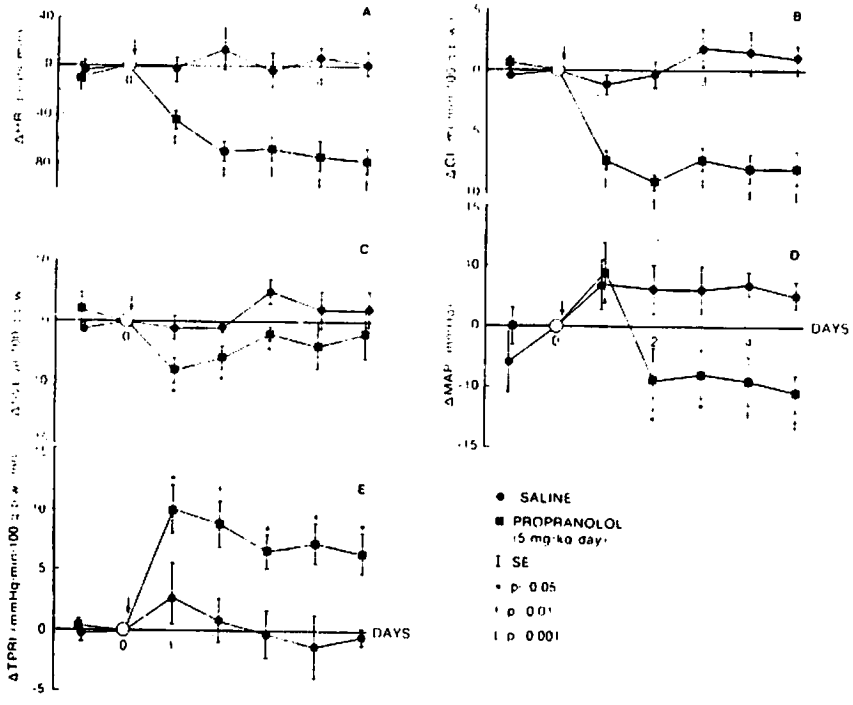

Figure 1 Effects of five-day subcutaneous infusion of saline or 5 mg/kg/day propranolol on (A) heart rate (HR), (B) cardiac index (CI), (C) stroke volume index (SVI), (D) mean arterial pressure (MAP), and (E) total peripheral resistance index (TPRI) in conscious SH rats. Data represent means ± SEM. Redrawn from (23) with permission.

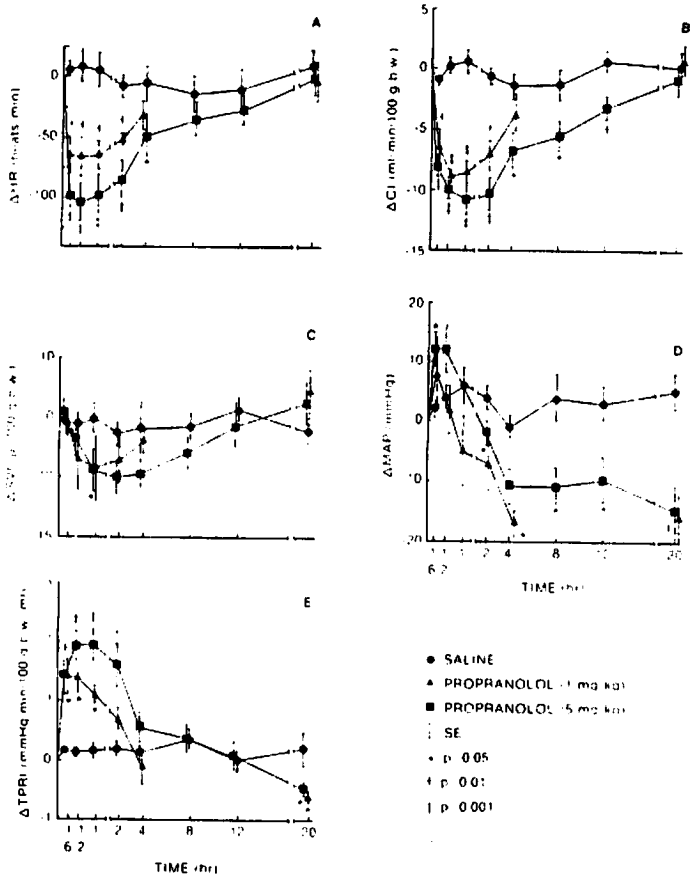

Figure 2 Effects of a single subcutaneous injection of saline, 1 mg/kg or 5 mg/kg propranolol on (A) heart rate (HR), (B) cardiac index (CI), (C) stroke volume index (SVI), (D) mean arterial pressure (MAP), and (E) total peripheral resistance index (TPRI) in conscious SH rats. Data represent means ± SEM. Redrawn from (23) with permission.

(Figure 2). As can be seen from Figure 1, it takes at least two days for the hemodynamic effects of propranolol to become fully developed and to produce the full extent of blood-pressure lowering. The constancy of hemodynamic parameters in the following days establishes that a steady state of response has been achieved after the second day of infusion. By contrast, the responses to a single dose (Figure 2) reveal a rather confusing picture, especially propranolol's clinically important hypotensive action. Using the multiday infusion method, Smits et al (24) resolved the previously conflicting evidence [see, for example, (24)] about the effects of this widely used drug in the SH rat and reconciled an important point of confusion about its validity as a model of human hypertension. The general point is that pharmacodynamic responses are not necessarily expressed completely, or even at all, within the time span of a drug's presence in the body after a single injection; continuing with the infusion until responses stabilize is basic to understanding the full range of a drug's actions.

Figure 3 shows the graded responses of urinary osmolality and volume on the

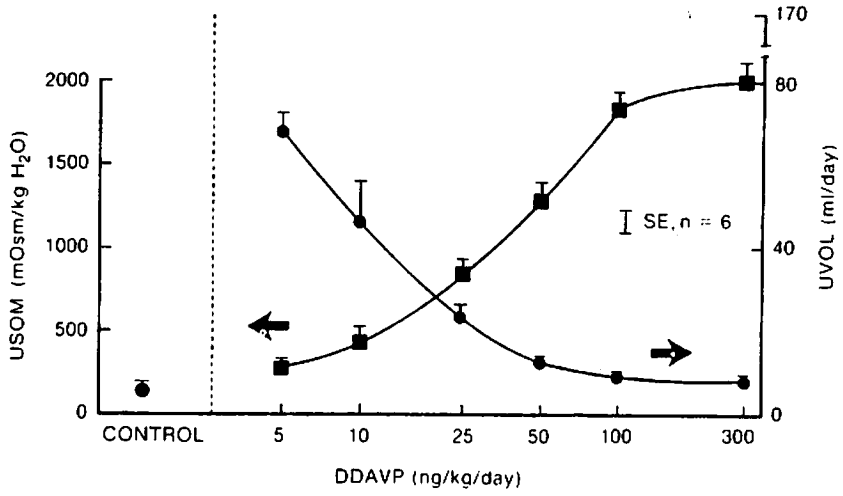

Figure 3 Responses of urine volume (U_{vol}, •) and osmolality (U_{osm}, ■) to increasing rates of infusion of dDAVP in six rats with congenital diabetes insipidus. Data were collected on the fifth day of infusion. Redrawn from (25) with permission.

fifth day of an infusion of deamino-8-d-arginine vasopressin in rats with hereditary diabetes insipidus (25). The infusion has made the duration of action of this peptide arbitrarily long; the agent's biological activity, in the sense of its bioassay, is defined by the slopes and variances of the two infusion-rate/response curves. Duration of action is usually regarded as a pharmacokinetic/pharmacodynamic attribute of the drug substance, but long-duration delivery systems make duration of action a pharmaceutical attribute. Multiday infusions at a series of graded rates, as in Figure 3, appear to provide a more precise way than conventional dose-response testing of defining an agent's pharmacodynamics, a theme that recurs subsequently.

SCHEDULE- OR REGIMEN-DEPENDENCE OF THERAPEUTIC INDEX: THE INJECTION-INFUSION COMPARISON PROTOCOL

Varying the schedule, regimen, or rate of administration has a major influence on the therapeutic index of some drugs; for others, regimen has little such influence. A recently devised protocol to test for this attribute calls for comparative dose-response testing when a drug is administered by a series of injections versus by infusions, each varied over the same range of total doses, given over a fixed time period. Sikic et al devised this protocol for studies of the pharmacodynamics of bleomycin (26); it has subsequently found application with other agents and appears to be a general protocol for basic pharmacodynamic assessment.

The studies by Sikic et al with bleomycin (017) are illustrative of the

injection-infusion comparison (IIC) protocol. These researchers administered bleomycin to tumor-bearing mice by three different five-day regimens, each covering the same range of total five-day dose: (*a*) an injection every 12 hours; (*b*) an injection on the first and third days only; (*c*) a continuous infusion by implanted osmotic pumps. The measure of efficacy was reduction in tumor size; the measure of toxicity was pulmonary fibrosis, assessed by hydroxyproline content of lung after bleomycin administration by the three regimens to non-tumor-bearing mice. At each of the higher total doses, the infusion regimen produced substantially and statistically significantly larger reductions in tumor size than did either injection regimen. In contrast, the infusion regimen produced substantially and statistically significantly less pulmonary fibrosis than did either injection regimen at equal total doses. The enhanced efficacy of the bleomycin infusion regimen has been confirmed (27).

The IIC protocol revealed that, in effect, the continuous infusion shifted the dose-response curve for bleomycin toxicity to the right while shifting the dose-response curve for its efficacy to the left. Thus, the therapeutic index of bleomycin in the mouse-tumor model was substantially widened by the use of the infusion regimen relative to that of either injection regimen. Subsequent clinical studies appear to confirm the extrapolation of this conclusion to humans (28, 29).

Recent work by Nau and his colleagues in Berlin (30–32) has added an important dimension to the use of the IIC protocol and the interpretation of its results. They have pointed out that many drugs have large interspecies differences in pharmacokinetics, usually with much shorter half-lives in small animals than in man; when these differences exist, rats or mice will have markedly exaggerated peak-and-trough patterns of drug concentration in plasma when drug is administered to them by once- or twice-daily injections, in contrast to much more modest fluctuations in drug concentrations in humans receiving once- or twice-daily dosing. In this circumstance, not only doses but also *dosing intervals* have to be adjusted in order to have bioequivalence between the regimen for the animal test and the regimen for human use. The work of Nau's group with valproic acid (VPA), described below, illustrates this point and its implications for the design of toxicologic tests: the terminal pharmacokinetic half-life of VPA is 8–16 hours in humans, but only about 0.8 hours in mice.

Nau et al (30) administered VPA by two different regimens in the same range of total doses to pregnant mice from day 7–15 of gestation, by once-daily injections and by constant-rate infusions from implanted osmotic pumps. With infusions, a substantially and statistically significantly higher total dose was required to produce both fetal resorptions and exencephaly than with the once-daily injections; fetal weight was adversely affected by both regimens, but the differences between the two regimens were small. In other words, the

dose-response curves for both fetal death and exencephaly were shifted to the right when VPA was given by infusion versus by once-daily injection; the dose-response curve for fetal weight loss was little affected by regimen.

In these studies, VPA concentrations in plasma during the infusions were essentially constant throughout the multiday study. With the injections, drug concentrations in plasma rose and fell quickly, and from about eight hours after each daily injection VPA was undetectable in maternal plasma until the next dose was given; then the cycle repeated itself. In humans, the once-daily therapeutic regimen produces VPA concentrations whose peaks are only about one-tenth as high as those in the mouse, and thereafter decline gradually to a trough level that is not less than about one-fourth that of the peak.

Scaling doses according to body sizes can provide equal time-averaged plasma concentrations in mice and humans, but the hour-to-hour concentration-time profiles differ drastically when VPA is given on the same once-daily basis to mice and to humans: much higher peak concentrations in mice, but much higher trough concentrations in humans. Running the IIC protocol with VPA in pregnant mice amounts to comparing the embryotoxicity of high but briefly maintained peak concentrations versus that of continuously maintained concentrations at far lower levels. Interpretation of the results should consider the fact that an infusion regimen that produces constant VPA concentrations in mouse plasma is much more nearly bioequivalent to the once-daily human therapeutic regimen than is a once-daily dosing regimen in mice.

The virtual absence of VPA during about sixteen hours of each day does not offset embryotoxic effects of injected VPA. With some drugs, such long drug-free intervals—"drug holidays"—may indeed minimize toxicity and show the injection regimen to advantage. With such evidence, it may be useful to study the effects of a combined regimen of daily injections superimposed on a constant infusion, with the infusions serving to maintain certain minimum concentrations at all times. Separate adjustments of the quantities administered by each mode can provide more or less independent control over the peak-and-trough concentrations. Gentamicin is an example of a drug whose toxicity appears to be less by intermittent injections than by constant infusions (33), but there are no animal studies on the effects of a combined regimen. Such a study might help resolve some of the controversy about the appropriate upper and lower limits of gentamicin concentration in human therapeutics.

The large difference in the pharmacokinetic half-life of VPA between mice and humans is not exceptional. The problem of interspecies size- and time-scaling has recently been analyzed by Boxenbaum (34, 35), giving examples of other drugs with large interspecies differences in pharmacokinetic half-life. These are important considerations in the design of pharmacologic and toxicologic studies on small animals, where bioequivalence to the human regimen may be crucial for valid extrapolation of the animal data to humans.

Endocrine Studies with the IIC Protocol

The effects of parathyroid hormone (PTH) on bone formation and resorption have been assessed with the IIC protocol. Tam et al (36) compared the effects of bovine PTH administered by daily injections versus continuous infusions in thyroparathyroidectomized rats. PTH infusion resulted in increased bone apposition and an increase in both bone formation and resorption surfaces, with a net decrease in trabecular bone. Equal doses by injection increased bone apposition rate and bone formation surface but did not increase resorption surfaces; in this case bone volume increased. The authors conclude that the injection regimen provides a way of separating the resorptive effects of PTH from its effects on apposition rate and that intermittent doses of the hormone would be more effective than continuous infusion in promoting anabolic skeletal effects.

Podbesek et al (37, 38) obtained similar results using the IIC protocol to administer PTH to intact greyhounds and found that the injection regimen increased trabecular bone volume while the infusion decreased it. Results with the injections were in agreement with those in patients similarly treated. The authors concluded that an intermittent dosage regimen, even though it only transiently elevates PTH levels, appears more promising than the infusion regimen for the treatment of osteoporosis.

In studies of hormonal feedback in the pituitary-thyroid axis, Connors & Hedge (39) compared injections and infusions of triiodothyronine (T_3) given in graded amounts to thyroidectomized rats. When continuous infusion maintained plasma concentrations of T_3 at slightly below normal levels, thyrotropin (TSH) levels were higher than in intact rats. At higher rates of T_3 infusion plasma concentrations of T_3 were elevated further, though still in the physiological range, and TSH levels were in the normal range. When the same doses of T_3 were given by twice-daily injection, TSH fell to low levels and reduced pituitary responsiveness to thyrotropin-releasing hormone to a greater degree than did the infusion. T_3 was a more potent feedback signal when given by injections, but the more nearly physiological responses were elicited by the infusion regimen.

The growth-promoting effects of human growth hormone (hGH) on hypophysectomized rats were assessed by injections versus infusions; for equal growth responses, injections required about 1.7 times more hGH than did infusions (40).

Conclusion

The IIC protocol appears to be an important new probe in pharmacodynamic testing. As the literature shows, it is not a foregone conclusion which regimen will prove superior for a given agent. The practical significance of the IIC protocol is increased by the variety of new methods for rate-controlled drug

administration to humans. If the infusion regimen proves distinctly superior in preclinical studies, there is increasing likelihood that constant-rate administration can be a practical option in therapeutics. Such was not the case a decade ago.

In toxicologic testing, the concept of interspecies bioequivalence in regimen design is new and clearly warrants broader study. A particular value of the IIC protocol may be uncovering toxicity-masking effects of drug holidays that are inescapable when short half-life drugs are given by once- or twice-daily injections. Novel delivery systems allow one to program the concentrations of test agents in plasma and other biophases of experimental animals and thereby equalize disparate pharmacokinetic half-lives. The IIC protocol is only a first step in that direction.

TISSUE MICROPERFUSION

Controlling the flow of drug solutions in the 0.5–1 μl/hour range brings a new way of localizing drug action: tissue microperfusion with drug solution via a fine-gauge cannula inserted directly into a specific tissue site, remote from the osmotic pump. Delivery systems that operate by diffusion do not provide a convective flow of drug solution and so can deliver drug only to their immediate surroundings. Thus, the delivery system and its contained drug has to be inserted into the very tissue that the experimenter seeks to modify. Sometimes that poses limiting problems, which microperfusion avoids.

Because their osmotic driving force is about 300 atm (8), the pumps are capable of delivering drug solutions into solid tissue or against arterial pressures of a few hundred millimeters of mercury without any measurable reduction of flow (41). Also, they can pump solutions directly into tissues through the smallest available cannulae and catheters without flow being impeded by the resulting back pressure. A biologically important point is that flows of 0.5–1 μl/hr appear to be low enough so that hydraulic damage and/or edema is minimal or absent in the microperfused region.

The first use of this method was to test the influence of norepinephrine on cortical neuronal plasticity. Kasamatsu and colleagues tested the hypothesis that norepinephrine regulates plasticity in a variety of ways in feline visual cortex. Microelectrodes measured neuronal firing patterns, allowing assessment of neuronal ability to change ocular dominance as vision was experimentally changed between monocular and binocular. Four important papers (42–45) from Kasamatsu's group established the following: *(a)* microperfusion of a field of visual cortical neurones with 6-hydroxydopamine (6-OHDA) eliminates the normal plasticity of early postnatal life; *(b)* microperfusion with norepinephrine of a field previously depleted of catecholamines by 6-OHDA restores the normal plasticity of early postnatal life;

(c) microperfusion with norepinephrine of a region of visual cortex in adult animals partially restores the plasticity of early postnatal life.

The stereotaxically placed cannula was connected to an osmotic pump implanted subcutaneously in the cervical region. The extent of lesion was less than 0.75 mm radius around the 26-ga cannula tip (43), whereas the extent of spread of radiolabelled norepinephrine occurred over a radius of 10 mm from the cannula tip (43, 44); the concentration of microperfused drug was always highest in tissue immediately adjacent to the cannula tip, declined exponentially, and asymptotically approached the background level at distances beyond 10 mm from the cannula tip. Measurements of neuronal electrical activity were routinely made at two mm from the cannula tip, well outside the area of mechanical (or chemical) lesion but near enough to the cannula tip so that the concentration of test agent was about two orders of magnitude higher than that found beyond the microperfused zone.

The 26-ga cannula used by Kasamatsu et al is unnecessarily large; it has an internal diameter (ID) of 254 microns. In microperfusion of rat hypothalamus with labelled amino acids, 30-ga needles (152 microns ID) were used satisfactorily (46), and in rat renal artery infusions plastic tubing with 100 micron ID was satisfactorily used (41). Perhaps a smaller cannula would have lessened the pericannular lesion. Also, Kasamatsu's group used isotonic saline as the perfusion vehicle, which is irritating, as will be apparent to anyone placing a drop of isotonic saline on the surface of the eye, in contrast to Ringer's or other solutions whose ionic compositions approximate that of extracellular or cerebrospinal fluid. Nevertheless, these methodologic fine points cannot detract from the conceptual and technical elegance of the experiments of Kasamatsu and his colleagues. The critical capability that opened up this new line of neurophysiologic/pharmacologic investigation is the nearly atraumatic, direct microperfusion of cortical tissue, allowing direct control over local tissue concentrations of test substances and straightforward within-animal control procedures.

Other laboratories have adopted Kasamatsu's methods, adding further information: Bear et al (47) showed that only microperfused but not systemically administered 6-OHDA is effective in reversing the neuronal plasticity of early postnatal life; Daw et al (48) confirmed Kasamatsu's findings and added the observation that plasticity in directionally sensitive visual cortical cells responds in a qualitatively similar manner to those involved in the monocular/binocular dominance shift; McCall et al (49) showed that chronic intracerebroventricular infusion (icv) of lysergic acid diethylamide influences binocular/monocular shift in the same way as does microperfused norepinephrine.

Mangano & Schwarcz (50) compared the size and nature of local lesions around cannula tips through which solutions of excitatory amino acids were

either injected or continuously microinfused. They compared two weeks of 12–hourly 0.5 μl injections with two weeks of continuous infusion of glutamic, aspartic, and cysteine sulfinic acids at 0.5 μl/hour into rat striatum or hippocampus. The injections produced lesions of about 0.5 mm radius around the cannula tip, whereas no lesions occurred with the infusions. The authors concluded that very efficient mechanisms for the cellular uptake of the amino acids exist for protecting neurones against their toxic effects but that the uptake processes appear to have been saturated by the injections, suggesting a model for the neuronal damage in Huntington's disease.

Other CNS microperfusion studies include one-week microperfusion of glucose into basal ventromedial hypothalamus, effecting a 27% reduction in food intake (51); angiotensin II into the olfactory bulb, causing increased nocturnal water intake (52, 53); amphetamine into the caudate-putamen, causing a form of behavioral toxicity that suggests the occurrence of a local lesion, although no demonstrable anatomic lesion could be found at the cannula site (54); dopamine into the nucleus accumbens, producing hyperactive behavior that persisted throughout the fourteen-day microperfusion and for several weeks thereafter (55–57); labelled amino acid precursors into rat hypothalamus (46, 58) and striatonigral tract (59).

Kroin & Penn (60) studied the spatial concentration gradients of cisplatin in brain tissue around a microperfusion cannula. They showed that a platinum concentration of >2 ng/mg can be maintained throughout a 1 cm diameter sphere of tissue. If all the measured platinum were in the form of cisplatin, the observed minimum tissue concentration would lie within the apparent therapeutic concentration range. Kroin & Penn suggested that the microperfusion technique might be used to treat tumors up to 1 cm in size, with multiple cannula-pump units required for larger tumors. Their suggestion warrants further research in animal models of human brain disease in which there are large unmet therapeutic needs, e.g. neoplasms and focal epilepsy, where problems of passage through the blood-brain barrier may be limiting and chemical structural modifications of drugs [see, for example, (61)] to achieve penetration of the blood-brain barrier may not be possible.

Another application of tissue microperfusion has been in the study of trophic influences of nerve activity on skeletal muscle. Drachman et al (62, 63) microperfused rat soleus muscle with α-bungarotoxin in order to effect continuous blockade of acetylcholine at the neuromuscular junction. Microinjection methods had been unsuccessful in getting either the completeness or the continuity of blockade needed to evaluate the role of acetylcholine in neurotrophic regulation of skeletal muscle. Microperfusion established persistent, complete blockade of acetylcholine transmission, evidenced by the complete absence of miniature end-plate potentials and the absence of both electro-

physiologic and mechanical response to motor-nerve stimulation. There was no evidence of either histologic or ultrastructural damage to motor-nerve terminals in the microperfused region. This technique allowed Drachman et al to show that blockade of acetylcholine transmission produces changes in resting membrane potential and extrajunctional acetylcholine receptors that are quantitatively equivalent to those of surgical denervation, both in respect to time course of onset and the extent of the fully developed changes. This work supports the conclusion that acetylcholine release, both impulse-related and spontaneous quantal and nonquantal release, is the neurotrophic influence and that loss of postulated other neurotrophic factors is not necessary to account for muscle atrophy after denervation.

Eliason & Maurice (64) described microperfusion of various parts of the rabbit eye, including the corneal stroma. Chappel and colleagues (65) used the microperfusion method to overcome a long-standing point of confusion about whether the site of estradiol feedback action is the anterior pituitary or the hypothalamus.

An important variant of the tissue microperfusion method is the implantation of drug-releasing ethylene vinyl acetate (EVA) copolymer pellets directly in tissue to be studied. The preparation of such pellets has been described by Langer & Folkman (9); they have been used by Folkman and his colleagues in studies of the biology of angiogenesis, the new blood-vessel growth essential to supporting the growth of tumors beyond the few millimeters through which their metabolic needs can be met by diffusion alone. Heparin delivered by EVA pellets is a promoter of angiogenesis and protamine is an inhibitor (66), but heparin or heparin fragments plus cortisone are profound inhibitors of angiogenesis, both locally and, in sufficient doses, systemically (10).

Another variant of the microperfusion method is to infuse blocking or anesthetic agents into special perineural cuffs that bring the drug solution into close contact with the nerve surface. In this manner, Lorkovic (67) obtained motor-nerve transmission blockade with lidocaine and marcaine for 3–7 days, with denervation-like supersensitivity to acetylcholine in muscle innervated by the blocked nerve. Betz and colleagues (68) used this technique with tetrodotoxin to block nerve conduction for 5–13 days without evidence of nerve damage in a study of the factors that can lead to the sprouting of active nerve terminals in muscle.

Another variant of the tissue microperfusion method is an arrangement for the local delivery of substances to the experimentally injured or transected spinal cord in rats (69). A PE10 catheter was positioned over a gelatin sponge pledget that in turn overlay the damaged region of spinal cord; the catheter was supplied by a subcutaneously implanted osmotic pump so that the infusate entered the gelatin sponge and thus had some manner of access to the injury site.

TOLERANCE AND DEPENDENCE

It is well known that repeated administration of many agents can produce tolerance, subsensitivity, or tachyphylaxis to the test agent; if the agent is withheld or if an antagonist is given, a withdrawal syndrome may ensue. It is widely assumed that continuous presence of the test substance will favor development of tolerance, subsensitivity, or tachyphylaxis. Hence, implantable drug delivery systems have found considerable use with a variety of agents in studies on the development or modulation of tolerance and dependence. Whether or not the assumption is valid that constant-rate infusion is always the surest path to tolerance is another question, one that might be advantageously tested with the IIC protocol.

A multiday infusion of sodium barbital in mice produced tolerance, as shown by a significant decrease in sleep time following a challenge dose of barbital 24 hours after cessation of the infusion; physical dependence was demonstrated either by responses to pentylenetetrazol or by convulsions elicited by handling (70). Tabakoff et al (71) infused phenobarbital icv in rats for 72 hours and then removed the pumps; 24 hours later a challenge dose of pentobarbital was given to demonstrate tolerance. Selective destruction of noradrenergic neurons by icv 6-OHDA or by specific lesions of the dorsal or ventral noradrenergic bundles prevented the development of barbiturate tolerance without altering the animal's response to acute barbiturate.

Wei & Loh first demonstrated that physical dependence is a concomitant of centrally acting opiate-like peptides (72). Using multiday infusions of the agents into periaqueductal grey, they resolved the previous confusion about the central actions of these agents that had arisen from contradictory studies with single central injections. Similarly, physical dependence to a whole series of opiate-like peptides has been demonstrated by Wei (73).

A chronically infused, ACTH-related peptide inhibited the formation of tolerance to infused morphine (74). Chronic spinal infusions have been used to demonstrate partial cross-tolerance between an enkephalin and morphine at the spinal level (75, 76).

Another variation in the use of implantable delivery systems in opiate receptor studies is the chronic exposure of animals to a test agent and the subsequent removal of organs from those animals to study mechanisms of tolerance and dependence. This is illustrated by studies from the Max Planck-Munich group of Schulz, Wuster, Herz, and others in which guinea pigs were continuously infused with specific opiates by seven-day delivery from implanted osmotic pumps; subsequently, the animals were sacrificed and the ileum of each was removed for in vitro organ bath studies of cross-tolerance (77, 78). Similar methods have been employed by the same group to study the development of differential tolerance and cross-tolerance in opiate receptors in other tissues (79–87).

Hetta & Terenius (88) demonstrated that multiday maternal infusion of naloxone significantly increased neonatal mortality. Moreover, the young exposed to naloxone in utero showed no significant analgesic response to morphine until 40 days of age.

Implanted pellets of morphine have long been used to maintain the continuous presence of morphine in experimental animals, but the uniformity of morphine-release kinetics has been an unknown since the pellets are made on a small scale in many laboratories. Also, the field of opiate receptor pharmacology now involves many agents besides morphine, not all of which are readily formed into slow-release pellets by simple methods. Thus, it is likely that kinetically validated delivery system implants will gradually replace drug pellets in quantitative studies of opiate receptors and their regulation, as has largely happened in the study of hormone-receptor regulation.

In studies of ethanol dependency, using delivery systems to maintain chronic icv infusions has permitted several new approaches. Volicer et al (89) studied the comparative effects of chronic icv infusion of calcium and a chelator on alcohol dependence and tolerance. Rigter et al (90) demonstrated that vasopressin fragments were capable of enhancing both ethanol dependence and its withdrawal syndrome. Tuomisto et al (91) showed that fourteen-day icv infusions of tetrahydro-β-carbolines in rats substantially increased voluntary alcohol intake, an effect that took more than a week to develop.

Finally, the development of tolerance to chronic treatment with many other agents, including dermorphins (92), d-amphetamine (93), phencyclidine (94), and α- and β-agonists (95) has been studied using periods of chronic infusion of the test agent.

Studies on Receptor Regulation

In this field, delivery systems have been employed to ensure the continuous presence of many different drugs, hormones, and humoral substances at various pre-selected levels. This approach has been used particularly to study receptors for angiotensin II and gonadotropin-releasing hormone (GnRH) as well as for other agents, as is summarized in Table 1.

RATE-CONTROLLED MODULATION OF HORMONE LEVELS

Two major uses of both polysiloxane capsules and osmotic pumps have been to provide a constant background of hormone replacement after removal of the endogenous source of hormone and to maintain continuously high hormone levels. The capsules have been mainly used with estradiol and other steroids for reasons stated earlier.

Knobil and associates (117) mapped the effect of different rates of estradiol

Table 1 Receptor regulation studies done by chronically exposing animals and tissues to test agents delivered via implanted delivery systems

Receptors	Agents delivered	References
Response is to increase receptor population (up-regulation):		
Adrenal angiotensin II	Angiotensin II	96–98
Uterine myometrium angiotensin II	Angiotensin II	99
Pituitary GnRH	GnRH (low dose)	100
Brain opiate	Naltrexone	101
Brain cholecystokinin	Haloperidol	102
Brain dopamine	Prolactin	103
Liver estrogen	Growth hormone	104
Testicular LH	Luteinizing hormone	105
Testicular prolactin	Luteinizing hormone	105
Hepatic prolactin	Growth hormone	106
Lymphocytes-adrenergic	Propranolol	107
Cardiac-adrenergic	Propranolol	107
Lung-adrenergic	Propranolol	107
Hepatic growth hormone and prolactin	Growth hormone	108
Response is to decrease receptor population (down-regulation):		
Pituitary GnRH	GnRH (high dose)	100, 109
	Buserelin	110
Ovarian GnRH	GnRH	111
Testicular LH/hCG	GnRH	112
Cardiac β-adrenoceptors	Isoproterenol and norepinephrine	113
	Isoproterenol	114
Brain α_2-adrenoceptors	Clorgyline	115–116

administration on this hormone's feedback control of gonadotropin secretion, revealing the essential quantitative features of how estradiol can act at times to inhibit gonadotropin secretion but at other times to stimulate it. This work laid the foundation for the subsequent discovery of the integrative mechanisms that control ovulation and the menstrual cycle in man and other primates (118). Having control over the rate, as well as the quantity, of estradiol administration, was essential in resolving the confusion inherent in the sometimes-negative, sometimes-positive feedback actions of estradiol.

Another artful use of steroid-releasing polysiloxane capsule implants is the work of Ewing et al, who showed how graded rates of testosterone release could progressively inhibit spermatogenesis in rabbits (119) and monkeys (120) via dose-rate dependent inhibition of pituitary secretion of luteinizing hormone (LH). In the rabbit, but not in the monkey, it was possible to define a rate of testosterone release that totally suppressed spermatogenesis; however, still higher rates of testosterone release produced dose-rate dependent stimulation of spermatogenesis via a direct testicular effect of the steroid (119–120).

Inability to achieve complete suppression of spermatogenesis in the monkey appears to result because the direct stimulatory effect of testosterone on spermatogenesis occurs at a testosterone dose-rate insufficient to give complete suppression of pituitary LH secretion (120), thereby thwarting the hope that rate-controlled testosterone administration might provide a basis for hormonal contraception in males. Subsequent work has focused on the combined effects of testosterone and estradiol on the inhibition of spermatogenesis (121–122). The paradoxical effects of testosterone only became definable and understandable when the hormone was administered in a rate-controlled manner.

In the absence of endogenous hormone, multiday, rate-controlled replacement at varying rates allows one to probe the adequacy of regimens of hormone replacement. Considerable work has been done with the peptide hormones, whose generally very short half-lives virtually mandate either continuous infusions or very frequent injections in order to achieve chronicity of action, and in some instances any action at all.

The infusion mode has been used to assess effective replacement rates of aldosterone in the rat (123); angiotensin II during captopril blockade in the rat (124–125); insulin in the mouse (126), rat (127), and hamster (128–129); and vasopressin in the Brattleboro rat (130–133). The euglycemic replacement rate of insulin normalized the uterine growth response to estradiol (134), ameliorated proteinuria (135), and normalized gut sterol synthesis (136) in diabetic rats. Hyperinsulinemia has been maintained for as long as eight months by successive osmotic pump implants in a study of sucrose- versus starch-fed rats (137); other metabolic studies (138–140) have used shorter-duration maintenance of hyperinsulinemia. Multiday infusions of aldosterone for 1–4 weeks produced supraphysiologic levels of this steroid and demonstrated its hypertensive effect (141–143) and associated changes of ion transport in arterial walls (141–142). Multiday infusions of glucagon in rats mimicked the effects on hepatic urea cycle enzymes of high-protein intake (144). Multiday infusions of growth hormone induced feminization of hepatic steroid metabolism in an infusion-rate–related manner in the rat (145–148) but to elicit this action by injections required three- or six-hourly injections, whereas twelve-hourly injections were ineffective (148).

Certain assumptions underlie the use of constant-rate parenteral infusion of a single hormone to substitute for an intact gland. With insulin, for example, none of the published studies utilize intraportal infusion to mimic the intraportal secretion of insulin. Intraperitoneal (ip) placement of the delivery system is an uncertain method of approximating intraportal infusion, for some of the released agent may be absorbed across parietal peritoneum into the systemic circulation or across visceral peritoneum into the portal; the ceaseless motions of the viscera invalidate any assumption of constant fractions of ip-administered agent being absorbed by the two routes.

Another assumption is the use of constant-rate replacement to approximate the function of those glands whose secretions have clearly established rhythmic patterns—infradian, circadian, supradian. Fortunately, the constant-rate osmotic pumps can be adapted to deliver test agents in a preprogrammed rhythmic pattern, as Lynch et al (149–150) have elegantly demonstrated with circadian variations in the rate of melatonin infusions (see below). However, evaluation of the significance of rhythmic patterns of hormone secretion/replacement remains an underdeveloped area of research with the notable exception of GnRH, whose physiologic actions depend so clearly on a pulsatile pattern of secretion/replacement (118). As noted earlier, Connors & Hedge (39, 151–152) compared the effectiveness of periodic versus continuous replacement of thyroid hormones as feedback inhibitors of TSH secretion.

Multiday infusions introduce a new type of hormone bioassay; the hormone's actions are assessed in a broader context and over a longer period of time than in the conventional single-dose hormone bioassay. For example, Patel's study (127) of insulin replacement regimens in streptozotocin-diabetic rats spanned 60–80 days, with repeated replacement of two-week duration osmotic pumps. Such experiments call for careful attention to detail, as illustrated by contrasting the design of Patel's study with that of Lopaschuk and colleagues (153), whose work virtually catalogs the technical problems that can confuse the assessment of continuous administration of a peptide hormone for even as short a time as seven days.

Technical Considerations

Important technical considerations in the rate-controlled modulation of hormone levels are: (*a*) to avoid infection at the implantation sites, a special problem in diabetic animals though rarely one in normals. Infected implantation sites may not be purulent and may only show serous or serosanguinous fluid between the implant and a surrounding capsule; (*b*) to formulate the vehicle so as to insure its physical and chemical stability within the delivery system throughout the infusion period; (*c*) to monitor physiologic responses to the infusions at reasonable intervals and to establish with objective evidence, rather than casual surmise, the basis for time-varying responses during a supposedly constant infusion.

With respect to the last point, there are now many proven examples of receptor down-regulation or fade during constant infusions (see above); however, an initially high response that subsequently fades during a presumed constant infusion may be due to: (*a*) decline in infusion rate due to changes in delivery system performance, instability of the agent within the reservoir of the delivery system, or binding of the agent to the delivery system reservoir or infusion tubing; (*b*) changes in pharmacokinetic clearance of the infused agent; (*c*) pharmacodynamic changes, such as receptor "fade." Documentation of the

plasma concentration of the infused agent is a logical first step in understanding which of these various mechanisms may be operating. Any peptide solution prepared for chronic infusion has to be regarded as an excellent microbiological growth medium and so should be formulated and handled with strict aseptic technique, with careful consideration given to including an established preservative or other anti-microbial agent in the formulation.

A special problem with insulin is the tendency for solutions of the hormone to aggregate within the reservoir of delivery systems; Grodsky and his colleagues have reported that the inclusion of dicarboxylic acids in the vehicle can prevent aggregation (154). A special problem with parathyroid hormone is its propensity to bind to polymeric surfaces, including those within the osmotic pumps. This problem can be prevented by siliconizing the pumps and any catheters used with them (155).

Use of Delivery Systems to Assess Biological Activities of Hormones and Hormonal Metabolites or Analogs

The use of continuous delivery as the basis for bioassay of estrogenic activities has been explored by Martucci & Fishman (156–157), who assessed the comparative uterotropic activities of 2- , 4- , and 16-hydroxylated metabolites of estradiol in the rat. In prior work, paraffin pellet implants had been used to provide multiday delivery, but Martucci & Fishman had found too much uncertainty in the kinetics of release from the pellets. Using implanted osmotic pumps, these workers showed a sustained uterotropic action of the 4- and 16-hydroxylated metabolites, a short-lived uterotropic activity of 2-hydroxyestradiol, and the ability of 2-hydroxyestrone to increase plasma LH concentrations. These results were confirmed by Ball et al (158–159). A noteworthy point about these multiday infusions of catecholestrogens was their ability to discriminate short-lived versus sustained uterotropic activity.

Naish & Ball (160) examined the effects of estradiol and of 2- and 4-hydroxyestradiol on lordosis behavior in ovariectomized rats, comparing a 2-bolus intravenous injection regimen versus a seven-day subcutaneous infusion from implanted osmotic pumps. For equivalent lordotic responses, it required about one-fiftieth or less the amount of estradiol with the infusion regimen compared to the 2-bolus regimen. For 4-hydroxyestradiol, which also had lordosis-inducing activity, the infusion dose required was about one-tenth that of the bolus regimen. The 2-hydroxy metabolite was without lordosis-inducing activity and did not manifest any inhibitory effect on the activities of either estradiol or 4-hydroxyestradiol. Jellinck et al (161–162) showed the infusion-rate dependent series of biochemical, hormonal, and behavioral effects of estradiol in comparison to those of the 2- and 4-hydroxy metabolites and their differential minimum effective rates for the same biochemical effects in different tissues.

Multiday infusion appears to be a more sensitive method to detect biological activity than the conventional one- or two-injection approach. Another example of this principle is the several hundred-fold greater sensitivity in the response of striatal dopamine receptors to prolactin given by 7–8 day infusion versus 4–6 days of daily injections (163).

The infusion mode may be an important means of testing peptide factors of cellular origin, whose often rapid inactivation makes almost mandatory the use of continuous methods of administration to give the fullest opportunity for expression of biological effects. Some such work has already been reported (164–166).

FETAL DRUG DELIVERY

Fetal hyperinsulinism has been induced in the monkey (167–168) and in the pig (140) by direct implantation of osmotic pumps in the respective fetuses. In both species, fetal growth was normal. Hyperthyroidism has been induced in fetal lambs by chronic infusion of T_3 from fetally implanted osmotic pumps (169–170).

LABELLING STUDIES

^{3}H-thymidine, bromodeoxyuridine, and other radiolabelled metabolic substrates are well-established tools for assessing various aspects of cell turnover. When such substances are administered by single injection, however, the extent of their ensuing incorporation into cellular constituents depends on the turnover of the structure of interest. When turnover is slow, a single injection may not suffice to give adequate labelling and so a schedule of chronic administration of the labelling agent is needed. Repeated injections are an obvious possibility, but the stress of frequent handling can have major effects (171). For these reasons, a number of workers have adopted the use of the continuous infusion technique, using implanted osmotic pumps.

Table 2 indicates the range of applications of labelling studies with ^{3}H-thymidine. The majority of the tissues studied showed low rates of cell turnover, but with the continuing presence of the labelled nucleotide extensive labelling was eventually obtained. In the multiweek studies, animals were reimplanted with fresh one- or two-week duration osmotic pumps.

Bromodeoxyuridine infusions for 1–3 weeks have been reported (179–180), but to date no one has undertaken a formal comparison of the osmotic pump implant with the compressed tablet implant, whose kinetics of release may be capricious and subject to minor variations in formulation and tabletting parameters, as has been observed with compressed tablets in pharmaceutical manufacturing.

Table 2 Tissue labelling after multiday infusions of ^{3}H-thymidine

Species tissue	Duration of infusion	Route	Greatest incorporation	Reference
Hamster/periodontal ligament	6 weeks	IP	47	172
Rat/9L brain tumor	34 hours	SC	43	173
Rat/mammary gland	5 days	IP	80 (young rats): 0 (old rats)	174
liver			53	
intestine			95	
Mouse/lung parenchyma	12 days	IP	40	175
Rat/kidney	7 days	SC	?	176
Mouse/corpus callosum	7 days	SC	40 (subependymal cells) 2 (glia)	177
Mouse/vomeronasal organ	12 days	IP	17	178

Direct-tissue microperfusions with labelled amino acids have demonstrated in vivo synthesis of gonadotropin-releasing hormone from the preoptic area of rat hypothalamus (46) opiomelanocortin from the periarcuate region of rat hypothalamus (58), substance P from rat striatonigral tract (59) and melanotropins from pars intermedia of the amphibian *Xenopus laevis* (181).

Direct-tissue microperfusion with labelled precursors is a powerful and potentially widely applicable method for demonstrating the in vivo synthetic activity of a tissue and, of course, circumstances of activated synthesis. The ability of the microperfusion method to maintain a localized pool of radiolabelled precursor/substrate within a few millimeters of the cannula tip (see above) suggests novel experimental approaches, especially in brain biochemistry.

DO DELIVERY SYSTEMS INCREASE OR DECREASE THE STRESS OF DOSING?

Parenteral administration of bioactive agents to experimental animals requires that the animals be handled, except when the agent can be given by inhalation of ambient air. Unless the animal is domesticated or has received prior training, handling induces tachycardia, acute release of several pituitary hormones—including growth hormone, ACTH, and vasopressin—and usually either aggressive or escape-directed behavior. Adding agents to food or drinking water are ways to avoid repetitive gavage and are used when it is technically possible to do so, and when the imprecision inherent in coupling dosing to

appetitive behavior is judged less important than the problems posed by frequent handling.

Delivery systems can reduce handling to once every one, two, or four weeks but they add the need for anesthesia and surgery. Subcutaneous implantation is a minor procedure in an animal that is large relative to the implant, but has to be considered more of a major procedure when the creation of a subcutaneous pocket for the implant involves a tenth or more of the animal's surface area, as it may in the smallest animals. Intraperitoneal implantation can be done through a keyhole incision, avoiding handling of the viscera, but if the peritoneal cavity is opened widely and the viscera handled, there will usually be one or more post-operative days of diminished food intake and deviation from the normal growth curve. Additional procedures, such as leading catheters from the implant to veins, arteries, cerebral ventricles, or the gut lumen, may lengthen the time until food intake is normal and body weight resumes its expected time course. The focus on food intake and body weight as integrated measures of surgical stress reflects clinical judgment above the close interrelations among well-being, appetitive behavior, nutrition, and growth.

Not all stress is surgical nor does all stress manifest itself in diminished food intake and impaired growth. Nontraumatic stress has unfortunately to be described in anecdotal rather than analytical terms. Handling of animals, subjecting them to frequent injections, or restraining them during infusions can all introduce stress into experimental protocols. The usual means of controlling the experiment is to administer only a vehicle to animals selected from the same group as those receiving the test agent, using the same regimen and procedures in both groups. This procedure reveals the effects of the test agent in stressed animals but does not eliminate stress. Therefore, stress is an unintended variable in many studies and is sometimes a confounding one also.

An illustrative and cautionary example is the work of Riley (171), who showed that the appearance of mammary tumors in female mice of the C3H He strain carrying an oncogenic virus was markedly accelerated by a variety of stressful factors, including frequent handling. Embryotoxicity can be enhanced by extra handling of pregnant mice (H. Nau, unpublished observations). A less obvious factor is the possible interaction between stress-related hormonal changes and the agent being administered.

Reported experiences with delivery systems in relation to stress are numerous, mostly anecdotal, but uniform in concluding that neither implantation nor other procedures accompanying implant use appeared stressful (46, 60, 69, 110, 132, 172, 182–183). Most of these studies lasted for 1–2 weeks, but several longer studies, with successive reimplantations, have been performed and assessed from the point of view of the stress involved. De la Torre & Gonzalez-Carvajal (69) have infused several agents to the spinal cord of rats via osmotic pumps for as long as sixteen weeks, reporting that there were no

evident adverse effects on the rats. Akhtar et al (110) reported normal body weight and no adverse effects in monkeys over a 20–week period during which these delivery devices were replaced weekly.

The most searching probe of the possible stress associated with implant use has been done by DeLuca and his colleagues in connection with their studies (183) on 20–week infusions of vitamin D metabolites in D-deficient female rats from weaning through a complete reproductive cycle and lactation. The animals were ether-anesthetized every two weeks for osmotic pump replacement. Some of the animals in the study received vitamin D metabolites orally on a thrice-weekly schedule. The maternal growth rates, pregnancy rates, birth rates, litter sizes, litter weights, pup survivorships, and pup weight gains during lactation were comparable between the orally treated and implant-treated animals and compare favorably with normal rats. The only observed difference was that half the animals receiving 25-hydroxyvitamin D_3 by implant had irregular estrous cycles versus one-fourth among those receiving the same agent orally; however, only one-fourth the group receiving the 1,25-dihydroxyvitamin D_3 by implant had irregular estrous cycles, so it is not evident that repetitive implantation alone had an adverse effect on estrous cycling. The authors concluded from the evident normality of growth, reproductive, and nurturing parameters that the removal and reimplantation of the osmotic pumps every other week "did not place a significant stress on the animals" (183).

It appears that delivery system implants can be used on a long-term basis with little or no adverse impact on the experimental animal from the presence of the implant or the procedures for placing it, provided the procedures are done with reasonable skill. It seems that delivery systems add little to the stress of dosing and may be a lower-stress alternative to frequent injections or gavage.

INTRA-ARTERIAL INFUSION

Smits and his colleagues in Maastricht have developed a method for chronic infusion into the rat renal artery (41). Despite the technical obstacles posed by the small size of this vessel and its branches, the rat offers the advantage of uniform pathophysiologic parameters within various strains, plus use of the SH rat model of human hypertension.

Obstruction of renal blood flow was avoided by cannulating the right suprarenal artery and passing the cannula retrogradely so its tip lay at the entrance to the right suprarenal from the right renal artery; the opposite end of the cannula was attached via a larger-bore catheter to an osmotic pump subcutaneously implanted in the neck. Clearance studies (41) showed that the procedures had no adverse effect on right renal function in chronically left-

nephrectomized animals during infusion with isotonic saline; the 1 μl/hour infusion rate contrasted with renal plasma flows of about 400 ml/hour.

The first published experimental work with this new method showed the hemodynamic consequences of renal arterial infusion of norepinephrine in normotensive rats: mild hypertension, elevated total peripheral resistance, and slightly diminished cardiac output—all dose-rate dependent—plus a dose-rate–independent, relative bradycardia (184). Many intriguing studies of nephrogenic reflex and humoral mechanisms in normal and SH rats are now possible, and one can anticipate many publications from both the Maastricht group and other laboratories that have adopted their method.

CIRCADIAN RHYTHMS AND OTHER PATTERNED DRUG REGIMENS

Either constancy or gradual decline of rate are characteristic of the four mechanisms used to date in implanted delivery systems: solution diffusion, osmosis, thermally generated vapor pressure, and dissolution. Hence, an additional mechanism is required in order to generate on-off or otherwise oscillatory patterns of release of active agent.

One ingenious solution to the problem of converting constant flow from an implanted pump into a circadian on-off pattern of drug delivery has been provided by Lynch et al (149–150) at MIT. To implanted osmotic pumps they attached a long, narrow-bore polyethylene catheter, carefully filled with an alternating sequence of vehicle and drug solution; the long catheter was formed in a tight coil so that when implanted it occupied about the same amount of space as the osmotic pump to which it was attached. The constant flow from the osmotic pump into the coil displaced the coil's contents from its opposite end, thus putting an alternating sequence of drug solution and vehicle into the animal. The MIT group have used this method to infuse melatonin according to a circadian pattern and have validated it by measuring the temporal patterns of urinary excretion of melatonin and a dye added to one of the two solutions sequenced in the infusion coil. Ewing et al (185) have used this method to produce 0.5 hour pulses of LH and other pituitary hormones every two hours, in order to mimic the pulsatile pattern of LH secretion in the rat (186).

Patterned administration of certain hormones has only recently been recognized to be fundamental to expression of their physiological activities. The work by Knobil and colleagues (118) with GnRH has introduced an entirely new concept to the dynamics of hormone action: using an on-off pattern of delivery, they demonstrated that the physiologic actions of GnRH, and attempts to mimic those actions in therapy, depend on the frequency and amplitude of its administration. Neither dose nor fixed rate provides a basis for

expressing this hormone's actions. Such results should stimulate the explorations of time-varied patterns in the administration of many other biological substances to determine the extent to which their actions depend on frequency or amplitude of administration.

In a somewhat different experimental approach to circadian rhythms, other investigators have employed constant-rate infusions to influence the characteristics of physiologic rhythms. For example, Nakagawa (187) infused insulin continuously into various regions of the brain to investigate the role of this substance and that of carbohydrate metabolism in the generation of the circadian feeding rhythm in rats. Other investigators have continuously infused anti-depressant drugs to study their influence on the rest-activity cycle of female hamsters (188–189); for example, multiday infusion of clorgyline lengthens the circadian rest activity cycle (189). Additionally, infusion of clorgyline or imipramine for two weeks was found to induce dissociation of many of the components of the circadian activity rhythm (189), suggesting that chronic use of agents of this nature can modify circadian frequency and/or coupling between circadian rhythms.

Thus, novel delivery systems bring new capabilities to the study of biorhythms. In addition to the programmed coil method of the MIT group, it is useful to recall that the combination of injections and infusions can in principle give quite varied patterns, though if injections have to be very frequent handling stress may be a problem. Future technical advances in delivery-system design may bring versatile rate-programming capability in implantable delivery systems—and indeed some intriguing research indicates that external magnetic fields may be one means of programming rate (190)—but complex rate programs are thus far a very minor theme in pharmacology, though a growing one in endocrinology.

ENVIRONMENTAL STUDIES

Doherty, Ferm, & Smith (191) have called attention to the utility of chronic infusions as a model for environmental exposures. They studied the toxicity of chronically infused sodium cyanide to mimic the metabolism of nitriles or other cyanogenic agents. They found a narrow, steep relation between infusion rate and the appearance of teratogenic effects in pregnant hamsters; the evident minimum effective rate for teratogenicity is 95% of the rate that produces uniform fetal death and significant signs of maternal toxicity. It is doubtful that an injection regimen could have revealed this narrow range in which cyanide is teratogenic but not lethal. This is not an academic point because of the relative constancy of cyanide release from some aliphatic nitriles and amygdalin [see, for example, (191)]. For analogous reasons, Johnson & Foulkes used cadmium

infusions instead of bolus injections to approximate better the time course of cadmium ingestion via the diet (192).

DELIVERY SYSTEMS IN PHARMACOKINETIC STUDIES

The measurement of the total clearance of drug is simplified when measurements can be made in the steady state attained when constant-rate infusion of drug has been maintained for longer than 4–5 times the drug's terminal half-life (193). This is a useful approach when studies on small animals preclude the multiple blood samples required for pharmacokinetic characterization of drugs administered by injection or other bolus mode, as illustrated by the studies of Betlach & Tozer on genetic variations in theophylline biodisposition in mice (194–195) and the study of the steady-state tissue distribution of propranolol and its metabolites in the rat (196–197). The infusion mode is also useful to detect and characterize relatively slowly occurring changes in drug metabolism, e.g. those due to diurnal variations (198), those due to autoinduction as seen during continuous administration of phenobarbital in the rat (199), or those due to autoinhibition, as occurs during continuous administration of haloperidol in the rat (200) or p-chloroamphetamine in the mouse (201). The infusion mode also simplifies the study of spatial gradients in drug concentration within the body (193), as illustrated by work in rats on propranolol in various tissues (196–197) and on gentamicin in perilymph and endolymph (202–203).

REBOUND PHENOMENA AFTER WITHDRAWAL OF ANTIHYPERTENSIVE DRUGS

Thoolen et al have developed an animal model for the study of the withdrawal syndrome that occurs upon ceasing chronic administration of clonidine and other centrally acting α-agonist drugs. In a series of papers (204–209) they have established that the abrupt cessation of a 12–day infusion of clonidine leads to a several-day period of tachycardia and to a peculiar cyclic variation of arterial blood pressure: 3–6 minute episodes of 30–50 mm Hg elevations in arterial pressure, 7–8 times per hour, for 24–36 hours after cessation of the drug infusion. These findings have been confirmed (210). The infusion rate used by Thoolen and colleagues reduced blood pressure in the SH rat but had no effect on blood pressure in normal rats, yet the heart rate and blood pressure responses after cessation of the infusion were similar in the two kinds of animal. Centrally administered morphine prevented the tachycardia but not the cyclic upswings in blood pressure; these were blocked by centrally administered oxymetazoline.

Azepexole had a withdrawal syndrome similar to clonidine (207); neither guanfacine (208–209) nor α-methyldopa (209) given in infusion regimens that

were equihypotensive to that of clonidine showed a significant withdrawal syndrome (208–209). The intraperitoneal administration of yohimbine on the twelfth day of infusion of clonidine or guanfacine produced tachycardia and cyclic upswings in blood pressure, along with diarrhea, shivering, jumping, and ptosis (209); only slight shivering and minimal changes in blood pressure occurred when yohimbine was administered on the twelfth day of α-methyldopa infusion (209). It took at least five days of clonidine infusion before yohimbine injection precipitated this withdrawal syndrome (209).

These studies provide a useful animal model for the human cardiovascular sequellae of abrupt withdrawal from chronic drug administration. It is another example of an important drug response that takes days to develop.

NEW ANIMAL MODELS

A frequent limitation in preclinical drug studies is the unavailability of suitable animal models of human disease. Chronic duodenal ulcers, for example, do not naturally occur in the common laboratory species, and while injected histamine has been widely used to stimulate the secretion of gastric acid, it is not a very effective ulcerogenic. Recently, however, Hosoda et al (211) demonstrated that a continuous subcutaneous infusion of histamine in an African rodent, *Praomys (Mastomys) natalensis*, successfully produced multiple ulcers. Responses were dose-related and at higher delivery rates some perforations occurred. In contrast, histamine given as a single subcutaneous injection was not ulcerogenic. The authors suggest that the histamine-infused *Mastomys* may be a useful new animal model for the study of peptic ulcer disease. In addition, a rat model of peptic ulcer disease is suggested by the work of Szabo (212), who induced ulcers by chronic infusion of cysteamine.

To discriminate between central and peripheral actions of β-blockers, Smits et al (213) infused propranolol into an intracerebral ventricle or subcutaneously in the unrestrained SH rat. A five-day icv infusion achieved brain concentrations that were approximately 100-fold higher than those achieved by a subcutaneous infusion at equal rates, whereas plasma concentrations were approximately the same; both regimens lowered blood pressure to about the same extent. When the icv infusion rate was reduced so as to produce brain concentrations equal to those produced by subcutaneous infusion, the plasma levels of propranolol were very low and blood pressure remained elevated. These results support the conclusion that the antihypertensive action of propranolol in SH rats is a peripheral, not a central effect. As discussed in the introduction, Smits et al also demonstrated unequivocally the antihypertensive action of propranolol in the SH rat, which is an important piece of evidence supporting the SH rat as a model of human hypertension.

Comparative studies with antihypertensive drugs in models of hypertensive

cardiac hypertrophy show a puzzling dissociation between cardiac hypertrophy and hypertension. Greenberg & Wilborn compared the effects of three-week infusions of clonidine versus propranolol on the cardiac hypertrophy (214) of the SH rat: clonidine normalized myocardial mass but propranolol had no effect, even though the rates of infusion of the two drugs gave equal reductions in blood pressure. The propranolol-infused rats showed focal areas of myocardial necrosis whereas no such changes were seen in the clonidine-infused rats, a finding that clearly warrants further investigation. The cardiac hypertrophy that accompanies hypertension induced by continuous iv infusion of norepinephrine in rats is prevented by concomitant β-blocker treatment but not by concomitant α-blocker treatment, despite the fact that blood pressure in the norepinephrine- plus phentolamine-infused rats was lower than normal (215). Perhaps these puzzling differences are relatable to the striking and paradoxical changes in myocardial adrenergic receptors that the FDA group (95) has observed with osmotic-pump infusion of α- versus β-agonists.

In another hypertensive model, continuous-delivery captopril was used to study the role of angiotensin II as a renal component in the maintenance of blood pressure and fluid volume in sodium-depleted rats (216). The complex role of the renin-angiotensin system in the genesis of hypertension after renal-artery constriction has been analyzed with continuously infused captopril in one- and two-kidney rats: hypertension is prevented by captopril infusion in two-kidney rats but not in one-kidney rats (217). Fluid restriction as well as continuous blockade of the converting enzyme is necessary to prevent hypertension when the renal artery is constricted in one-kidney rats (218).

Experimental hypertension has been induced by multiday infusions of norepinephrine, infused intravenously (215, 219–221) or at much lower rates into the renal artery (184).

Mills et al (222) developed a hypoprolactinemic rat model, suppressing endogenous prolactin secretion by the continuous intraperitoneal infusion of lergotrile mesylate.

The X-linked hypophosphatemic mouse is a model of human vitamin D-resistant rickets. Normalization of the bone defect was possible with four-week infusions of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] provided the regimen was commenced in young animals (223); the vitamin D metabolite acts on intestinal absorption of inorganic phosphorus but the genetic defect in renal phosphorus excretion is unaffected (224). By comparing the effects of injections versus infusions of $1,25(OH)_2D_3$, Hefti et al (225) concluded that this vitamin D metabolite plays an important role in calcium metabolism but is not involved in its hour-to-hour regulation. The physiologic role of $1,25(OH)_2D_3$ has been assessed in two important studies by DeLuca and his colleagues (183, 226), who infused this metabolite and several of its analogues as the sole forms of vitamin D for 20 weeks through a complete cycle of reproduction and

lactation from the time of weaning in vitamin D-deficient rats. The results indicate that 1- but not 24-hydroxylation is required for growth, reproduction, and skeletal mineralization. This work resolves prior confusion about the physiologic role of $24,25(OH)_2D_3$ that arose from experiments in which the short half-lives of the vitamin D metabolites were not taken into account but which use of the delivery system in the most recent studies has obviated. A model for fetal-growth retardation in utero in the rat was developed by Gruppuso et al (227) utilizing subcutaneously implanted osmotic pumps to produce maternal hyperinsulinemia. Previous models had relied on maternal dietary restriction, uterine vessel ligations, or other surgical interventions. Chronic maternal hyperinsulinemia was achieved by continuous infusion of insulin, thereby decreasing the supply of glucose to the fetus in a regulated manner and reducing fetal growth.

Finally, as pointed out in other sections of this review, the use of implanted delivery systems has simplified greatly the chronic infusion of bioactive agents into the central nervous system, either icv or by direct microperfusion of specific brain regions. Implanted delivery systems obviate the need for special restraints that, together with chronicity of drug delivery, greatly facilitate studies of drug and hormonal effects on appetitive (228–235) and other behavior (91, 236).

CONCLUSION

Multiday constant-rate drug administration has considerable research utility as both a pharmacodynamic probe and as a pharmacokinetic equalizer of disparate half-lives. This mode of administering bioactive agents was impractical for so long that its utility has been largely ignored in both drug and hormone research. The advent of novel drug delivery systems at both the research and clinical levels has removed that obstacle and has opened the way to research on programmed-rate administration of bioactive agents. With multiple regimens made practical, identifying the one most nearly optimal for research or therapeutic uses of each agent is both an opportunity and a challenge.

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