— INSTRUMENTATION & TECHNIQUES —

A subcutaneous intracerebral drug delivery device for use in rhesus monkeys

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This large-animal intracerebral drug administration unit is designed to allow the delivery of drugs or other agents to discrete loci within the brains of animals while maintaining sterile conditions. It is an improvement over existing designs because it (1) maintains an absolute minimal dead space within the system, (2) is smaller in diameter (by approximately 80% than existing shunt catheters, minimizing tissue damage during placement, (3) is easily secured and requires minimal clearance over the cranium, and (4) maintains a sterile seal between the brain and periphery. Preliminary studies indicate that the device is well accepted by monkeys and is fully functional for periods up to a year. The device is intended for permanent implantation.

The ability to assess the effects of drugs and other agents on the central nervous system (CNS) has aided researchers both in the analysis of behavior and in pharmacology. Typically, this assessment is accomplished by means of intracerebral or intracerebroventricular (i.c.v.) drug injections. Such methods bypass the blood-brain barrier, peripheral degradative enzyme systems, and other conditions hostile to chemical structures, allowing direct assessment of effects on the CNS. Most i.c.v. studies have been done with rodents, in which case sterile conditions are less critical to the longevity of the preparation or to the health of the organism. Nonhuman primates and most other species are more susceptible to infection than rodents. Several devices have been engineered for i.c.v. use in primates over the years, but they suffer from potential exposure of CNS tissue to external elements. More recently, systems used successfully in the treatment of human infant hydrocephally have been adapted to deliver drugs in primates and other species. For example, we have used a modified Heyer-Schulte intraperitoneal shunt for i.c.v. drug delivery in rhesus monkeys (Glowa, Herkenham, Bacher, & Gold, 1991; McCully, Balis, Bacher, Phillips, & Poplack, 1990). These systems are well accepted by monkeys as they are long-lasting and require minimal maintenance. However, in such systems a sub-

cutaneous port is typically employed for convenient access, and it can be located at a substantial distance from the site of drug delivery. Drug delivery through such systems can be problematic if agents tend to adhere to the cannula material, or if the relatively large vehicle volumes required to flush the dead space cannot be tolerated. The current device was developed specifically to allow microliter volumes to be administered with a minimum of dead space within the systems. It maintains many of the advantages of these earlier preparations, while at the same time minimizing dead space and injection volumes. A description of the cannula and data concerning its function are presented.

METHOD

Fabrication

Figure 1 illustrates the main features of the cannula and its specifications. The device was fabricated from stainless steel stock and commercially available stainless steel hypodermic needle tubing. The flange and seal retainer were made in one piece from Type 304 stainless steel. The elliptical flange shape and the notches for retaining screws were cut with a computerized milling machine. The seal-retaining groove, the 0.156-in. diameter and the 0.437-in. diameter, and the hole for the cannula tubing were machined on a lathe. The cannula tubing was made from Type 304 stainless steel hypodermic tubing. The 0.025-in. diameter holes were milled and then electropolished to remove burrs. The distal end of the tubing was closed by burnishing it in a lathe. The tubing was press fitted into the flange body and silver soldered for strength. Various modifications of this basic design have been explored, including changes in the spacing of the holes in the tip of the cannula (see below, Figure 3), beveling the inner access to the cannula (to make entry with the needle easier), and routing out one side of the mounting-screw

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Figure 1. Drawing of the main features and specifications of the cannula. (See text for materials and construction details.)

holes to allow better access to the cranium for screw placement by twisting the cannula on its horizontal axis. We have found this alteration helpful because it is more difficult to drill the screw holes through the retaining plate than to mark them, rotate the plate and drill the holes, and then rotate the plate back. We have not noted any damage to cortical tissue if the rotation maintains the vertical axis of the cannula. The bevel aids in finding the cannula barrel when the needle is pushed through the septum.

The septum allows repeated injections through the skin and a seal to permit the surgical closure of the point of access to the cannula (i.e., the cannula entry is subcutaneous). The septum was purchased from a chromatography supply company (Sopelco) and press-fitted into the canister after final construction of the cannula assembly. It is designed to allow surgical replacement with the cannula in situ. The old septum can be removed with a pair of forceps after an incision, and its replacement can then be pressed into place.

Animals

Two adult male rhesus monkeys (*Macaca mulatta*), previously adapted to chair restraint and weighing from 7 to 9 kg, were used. The monkeys were individually housed in a temperature-controlled room ($23^{\circ}\pm 2^{\circ}\text{C}$) with a 12:12-h light:dark cycle (lights on at 6:00 a.m.) with free access to water.

Surgical Procedure

The sterilized cannula was inserted aseptically under isoflurane anesthesia with the use of a Kopf stereotaxic apparatus. The skin and periosteum were incised and reflected with a periosteal elevator to expose the cranium. A burr hole (4 mm) was made in the cranium with a Hall drill, 18 mm anterior and 2–3 mm lateral to stereotaxic

zero. To maintain immobility and orientation, the cannula was vertically mounted on a length of stainless steel syringe tubing attached to the stereotaxic instrument. The cannula, with a 3–4 ft section of clear sterile plastic tubing fit over the distal end of the syringe tubing, was lowered approximately 25 mm until entrance into the ventricular space was detected by an unrestrained influx/efflux of saline/cerebrospinal fluid. Once in place, the cannula was rotated 90° on its vertical axis in order to tap holes in the cranium for two stainless steel orthopedic screws. The cannula was rotated back in position so that the screws could lock it into place, the screws were turned to fully immobilize the cannula, and methyl acrylate cement was then used to permanently secure the cannula to the cranium. The cannula was flushed daily for the 1st week after implantation and at least once weekly thereafter.

We have found that both prophylactic treatment with penicillin before surgery and a flushing of the cavity created for the cannula canister with sterile saline before suturing are effective in preventing infection.

Confirmation Procedures

Three methods were used to assess the continued viability of the cannula. First, angiotensin II (human; Bachem, Torrance, CA) was administered through the cannula (100 pmol/kg, in 100 μl) and water consumption during a 1-h period was compared with water consumption when the vehicle was infused. The monkeys were adapted to primate restraint chairs equipped with 1-pint water bottles within easy reach. The effects of angiotensin were determined after several days' assessment of baseline consumption.

The effects of corticotropin releasing hormone (CRH) were also assessed on a food-maintained operant behavior. CRH is a hypo-

thalamic-pituitary neurohormone that has potent appetite suppressant effects (Glowa, Barrett, Russel, & Gold, 1992). The current assessment provided comparisons with effects found in other studies, as well as effects found in our laboratories (Glowa, Barrett, et al., 1991; Glowa & Gold, 1991; Glowa et al., 1991) with the use of a subcutaneous port/catheter i.c.v. system (described earlier) in monkeys. Briefly, the monkeys were seated in a Plexiglas chair (Plas-labs) during the experimental sessions. The chair was placed in a ventilated, sound-attenuating chamber (Industrial Acoustics, Model AC-5) provided with white noise. A response lever (BRS/LVE, Model 121-05), modified with a solid Plexiglas paddle, was mounted on a Plexiglas panel affixed to the far wall of the chamber. Each press on the lever with a minimum downward force of 0.20 N produced an audible click of a relay within the chamber and was recorded as a response. Blue and red lamps, mounted at eye level behind the front panel, could be illuminated to serve as visual stimuli. A food-pellet dispenser (BRS/LVE, PDC-050) was mounted in the chamber. One-gram banana-flavored food pellets (Noyes) could be delivered to a tray that was accessible to the monkey through an opening in the front panel. The experimental conditions were controlled through a 64K networked experiment controller system (Palya, 1988), and cumulative recorders were used to monitor behavior on line.

The monkeys were previously trained to respond for food pellets under various schedules of reinforcement. Under the present conditions, responding was maintained under a fixed-interval (FI) 1-min schedule of food-pellet delivery until stable. Under this condition, in the presence of red stimuli, the first response after the elapse of a 1-min interval produced a food pellet. A maximum of 10 food pellets was available during a series of FIs. Typically, a session (approximately 60 min) consisted of four series of 10 FIs, separated by a 5-min time-out. During a time-out, the stimuli were not lighted and responding had no scheduled consequences.

For drug administration through the cannula, sterile conditions are maintained by (1) keeping the area directly over the cannula shaved (approximately 3 in. in diameter), (2) scrubbing with alcohol-betadine-alcohol swabs, (3) using sterile Huber point needles (such needles minimize "coring" of the septum and are available from several sources), and (4) using micropore filters to sterilize the solutions. The injection needle is simply pushed through the skin at the site of the cannula and then through the septum. For repeated daily injections, the scalp is moved to prevent the use of the same site. Ovine CRH (Bachem, Torrence, CA) was given as a dose of 1 $\mu\text{g}/\text{kg}$ in 100 μl of sterile saline, 60 min before experimental sessions. CRH or vehicle was then given as a single bolus dose, and the effects were compared with performances on the preceding day.

Figure 2. The effects of angiotensin II on water consumption. Non-water-deprived monkeys ($n = 2$) were allowed free access to water while chaired. Normally, little water (10 cc) was consumed over a 1-h period. Following the i.c.v. administration of 100 pmol/kg of angiotensin, water consumption increased to approximately 300 cc.

Figure 3. The effects of 1 $\mu\text{g}/\text{kg}$ of CRH on schedule-controlled responding. Abscissa: Condition (control compared with mean effect in 2 monkeys). Ordinate: Effect, as a percent of control (results are the average from 2 monkeys).

To further confirm the efficacy of the cannula, the monkeys were anesthetized and placed in a stereotaxic instrument with off-set ear bars. An X-ray was initially taken from the lateral aspect and then 1 cc of air was slowly infused through the cannula and another X-ray was taken. A third X-ray was taken after another 1 cc of air was slowly infused into the lateral ventricle.

RESULTS

Figure 2 shows that angiotensin significantly increased drinking ($p < .005$). The effect began within the first 2–3 min after administration and lasted approximately 20 min. The monkeys did not otherwise appear to be different from normal.

CRH decreased responding for food in a dose-dependent manner. Figure 3 shows that 1.0 $\mu\text{g}/\text{kg}$ decreased responding to approximately 32% of control levels.

Figure 4 shows a positive image of the X-ray taken after air was injected into the ventricles. The illustration shows two monkeys (Q72 and O57). In the top frames (before air), the i.c.v. cannula are clearly visible but the ventricular space is not. In the middle (1 cc of air) and bottom (2 cc of air) frames, the ventricular space is clearly visible and is not further enlarged by 2 cc of air. The figure illustrates two different variants of the cannula, with different spacing of the holes in the cannula shaft. These modifications were made to accommodate differences in the depth of the ventricular space in different-sized monkeys. In both only the middle holes are directly adjacent to the ventricular space, suggesting that, with further experience, more precisely located spacing can be obtained. However, both versions of the spacing produced comparable results on all the tests, suggesting that placement of additional holes outside the ventricular space does not detract from the operational properties of the cannula.

DISCUSSION

This large-animal intracerebral drug administration unit is designed to allow the delivery of drugs or other agents to ventricular spaces (or easily modified for delivery to discrete loci) within the brains of animals while maintaining sterile conditions. It is intended for use in awake animals, and it is a considerable improvement over existing

Figure 4. X-rays taken before and after air was injected into the ventricles of 2 monkeys (Q72 and O57). In the top frames (before air), the i.c.v. cannula are clearly visible but the ventricular space is not visible. In the middle (1 cc air) and bottom (2 cc air) frames, the ventricular space is clearly visible and does not seem further enlarged by 2 cc air.

designs for the following reasons: (1) It maintains an absolute minimal dead space within the system. This is important because the dead space of a system requires total flushing to remove all drug or agent from the system, and larger injection volumes may be impractical or dangerous to brain tissue if their volume exceeds mechanical limits of the tissue. Limited dead space also ensures that the total dose is delivered to tissue. (2) The current device is smaller in diameter (by approximately 80%) than are existing shunt catheters, minimizing tissue damage during placement. (3) The current device can be secured easily, and it requires minimal clearance over the cranium. This

is important because previous devices were bulky, and perhaps uncomfortable as well as irritating to the surrounding tissue. Primates are particularly sensitive to objects on their heads and will pick at them. This greatly increases the likelihood of infection. (4) Most importantly, the current device maintains a sterile seal between the brain and periphery, and it does not require an external access (access is subcutaneous), preserving the integrity of the organism against external infection. Preliminary studies have indicated that the device is well accepted by the monkeys, and that it has remained patent and fully functional for 6 months. Angiotensin-induced drinking has not been

reported previously in the monkey, but the effect of this peptide was similar to that observed earlier in the rodent (Epstein, Fitzsimmons, & Rolls, 1970). This test not only demonstrated that the cannula was patent, but that gliosis had not infiltrated the tip to render diffusion difficult. Substantial CRH-induced decreases in food-maintained responding occurred at a dose that was previously shown to produce small but observable effects in the monkey (Glowa et al., 1992; Glowa & Gold, 1991; Glowa et al., 1991). This suggests that decreased dead space or surface adherence resulted in a larger delivered dose than with previous devices. The X-ray also confirmed patency. This method may be the easiest to use in many situations if X-ray facilities are readily available. However, we found the angiotensin-induced drinking to be of suitable rigor.

The cannula can be used for continuous delivery if access to the head area is denied and suitable sterility is maintained. We have attached PE tubing to a needle and used a battery-operated pump with a bacteriostatic ointment placed around the site of penetration.

In summary, the presently described device performs well and has enjoyed repeated functional success to date. It benefits from being relatively simple in design. One potential disadvantage is that access requires close contact with the head. Drug delivery can be continuously ar-

ranged during behavioral sessions if monkeys are prevented from gaining access to their heads. Thus, the device fulfills all expectations for which it was designed and could be easily modified for use in either smaller or larger animals.

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(Manuscript received July 8, 1991;
revision accepted for publication February 18, 1992.)