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Intraventricular Angiotensin Elicits Drinking in the Baboon[1] (40720)

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Introduction. It is well documented that the administration of the octapeptide, angiotensin II (A-II), elicits drinking by animals. This was originally demonstrated when A-II was given intravenously to rats (1) and since has been demonstrated in a large number of species (see reviews (2–4)). More importantly for the present report, A-II has proven effective as a dipsogen when administered intraventricularly (IVT) and at lower doses than when given peripherally (5, 6). There is one report that A-II elicits drinking by rhesus monkeys (7) and a suggestion that it may also serve this role in humans (8). One of the purposes of the present experiment was to extend these observations to another primate, the baboon. A second and more important purpose was to determine the ability of A-II to elicit drinking by the same animal on a number of different occasions spread over a several-month interval. The practical value of such a regimen is that A-II administration might then be used to determine the success of placement of intended IVT cannulas.

We found that (a) IVT A-II reliably elicits drinking by the baboon, (b) the effect persists over time and repeated injections although the magnitude of the response is diminished, and (c) the IVT administration of A-II provides a feasible and practical method for the *a priori* determination of the placement of potential IVT cannulas in this species.

Methods. Subjects were seven adult male baboons (*Papio cynocephalus*) obtained from and maintained by the Regional Primate Center of the University of Washington. The animals were maintained in standard primate restraining chairs (9) in sound-attenuating chambers (two or three animals/chamber) during an experiment (1 to 2 months) and in standard individual cages during the interexperiment intervals (1 to 2 months). All of the animals were subjects in an experiment determining the effects of the chronic administration of insulin upon food intake and body weight (10). Since each experimental protocol lasted several weeks, it was desirable to know before an experiment began whether or not a particular cannula had access to the ventricles.

After an animal was adapted to its chair, an apparatus containing six cannula guide sleeves (25-gauge) was surgically implanted onto the skull. The guide sleeves were cut so that their tips would lie approximately at the dorsal surface of the dorsal horn of the lateral cerebral ventricles (three on each side, placed at 16, 19, and 22 mm anterior to the interaural line). Within each guide sleeve, a 32-gauge obturator remained in place except as noted below for injections and infusions. Details of the apparatus affixed to the skull and the surgery have been described elsewhere (11).

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When an experiment was to be initiated, an injector cannula was inserted into one of the six sleeve guides. Because of the duration of the ensuing experiment, an *a priori* method for determining whether or not the tip of the cannula actually had access to the ventricle was needed. Ideally, we could have applied negative pressure to the cannula when it was in place and obtained a sample of cerebrospinal fluid (CSF). Unfortunately, this procedure proved too unreliable in a chaired baboon. We therefore adopted a procedure of administering A-II through the cannula when the baboons were water-replete and determining the effect upon water intake over the subsequent 30 min. Animals were always tested from 8 AM to 8:30 AM. They had had *ad lib.* water all night prior to the test. Food was made available after the test (8:30 AM).

When a cannula was to be inserted, the baboon was tranquilized with ketamine-hydrochloride (5 mg/kg, intramuscularly). An obturator was removed and a cannula (32-gauge) the same length as the guide sleeve was inserted. The cannula was attached to PE-10 tubing and a remote syringe filled with sterile synthetic monkey CSF (12). An initial attempt at placement verification was made by injection of 200 μl of CSF over a 15-sec interval. If no apparent leakage occurred (frequently, CSF came up the guide sleeve around cannulas not terminating within the ventricular lumen), the cannula was left in place and the attached tubing passed through a small opening in the wall of the chamber and attached to an infusion pump set for the chronic delivery of 1.33 ml of CSF/day. If leakage occurred, a cannula which was 1 mm longer was inserted instead and the procedure repeated. The cannula length (or the guide sleeve used) was changed in this manner until a cannula was successfully in place as determined by the absence of leakage.

On the day of the A-II test (usually 2 to 4 days following the insertion of the injector), 5 μg of A-II in 0.5 ml of CSF was injected through the cannula over a 30-sec interval. The injection was made from outside the chamber. The bolus of A-II solution was followed by a 0.7-ml bolus of CSF alone. The dose of A-II (approximately 500 ng/kg) was comparable to that used in other species (5, 6, 13). A control injection occurred on either the day before or the day following the A-II injection. For this, the same procedure was followed except that only CSF was in both the 0.5- and 0.7-ml injections. The difference of water intake over the subsequent 30 min between the two days was the dependent variable of interest. The order of administration of CSF alone or CSF plus A-II was random. Data were analyzed with the paired t test.

Results. The results following the initial administration of A-II to each animal are summarized in Fig. 1A. Administration of CSF alone was followed by an average water consumption of 17 ml over the 30 min. The average response elicited by the A-II was 294 ml of water. The difference was significant ($P < 0.001$) and manifest in every animal. The smallest increase of water intake relative to the control day following the initial administration of A-II was 170 ml. There were no differences observed between different cannula placements.

A closed circuit television system allowed noninvasive monitoring of the behavior of the baboons in the chamber during these tests. We were thus able to determine the latency to drink following the onset of the A-II injection. This was defined as placement of the mouth over the drinking spout since water intake per se could not be observed. Latencies to drink following the administration of A-II ranged from 10 to 50

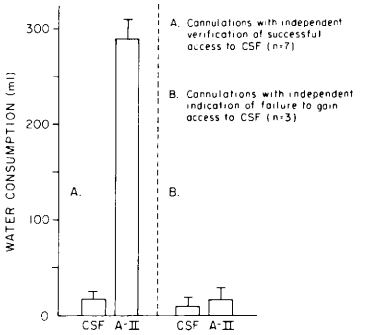

FIG. 1. Water consumption (ml) of baboons given either CSF alone (CSF) or CSF plus 5 μg of A-II (A-II). The brackets indicate the standard errors of the means.

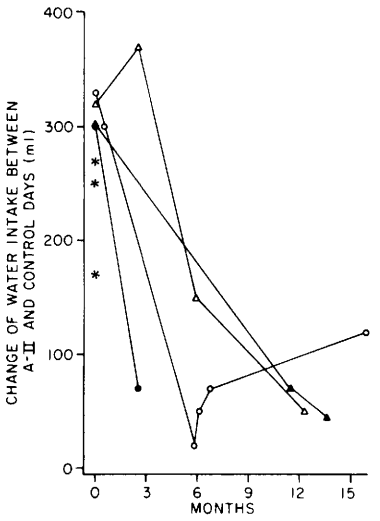

FIG. 2. Change of water intake (ml) between injections of A-II and injections of control solution into the cerebral ventricles of baboons. Values plotted are A-II day response minus control day response. Each point represents the response for one baboon, with the lines connecting the different A-II tests for the four animals which had repeated tests over the designated intervals. The asterisks represent the responses of animals given only one A-II test.

sec; latencies after CSF alone ranged from 40 sec to 22 min. Animals typically drank continuously for several minutes (up to 10) after the administration of A-II and then drank no more.

Figure 2 depicts the drinking responses elicited by A-II injection of individual baboons over time. The points at time zero represent the responses of the animals the first time they were given A-II and are the same data summarized in Fig. 1A. The later points represent subsequent cannulations in the same animals in which an identical procedure was followed. These later cannulations were necessitated by the occasional disruption of the CSF line by the animal and by the periodic cage rests. As can be seen, later administrations of A-II typically resulted in smaller yet still reliable increases of water intake relative to the control injection. Control intakes also decreased over the same interval such that most later control intakes were less than 10 ml. The smallest difference between A-II and control injections was 20 ml.

The nature of the specific chronic experiment on insulin infusion into the ventricles provided an independent verification of the success of placement of a particular cannula (10). Basically, that experiment showed that the infusion of insulin at doses of 10 or 100 μU/kg/day resulted in a decrease of daily food intake and body weight over a 2-week interval. For three such experiments, the infusion of insulin at those doses had no effect, whereas for the majority of experiments, a reliable, nonambiguous decrease of food intake occurred (10). For every point depicted in Figs. 1A and 2, the subsequent insulin infusion elicited a decrease of food intake. The A-II injection results obtained prior to the three negative experiments are depicted in Fig. 1B. There was no reliable difference of water intake between the A-II and the control days ($P > 0.05$) for these experiments. However, there was also no backflow when we initially tried to determine the success of cannula placement. Therefore, increased drinking following the administration of A-II prior to an experiment appears to be a practical method for determining the success of ventricular cannulation and is more reliable than either the presence of CSF or the lack of backflow in the cannula.

Discussion. These results demonstrate that IVT A-II is dipsogenic in baboons. The first time these animals were given A-II, they responded with a large increase of water intake (cf. Fig. 1A). Further, the latencies to drink were comparable to those reported for other species (5, 7, 13). These findings therefore extend to the baboon the finding that A-II administered into the cerebral ventricles elicits drinking.

These results indicate that the administration of A-II through a cannula aimed at the lateral ventricle of a baboon is a practical method for the *a priori* determination of the success of placement. In each instance for which we had independent evidence that a particular cannula placement was patent, the animal had previously had a positive drinking response to A-II. Likewise, for those few instances for which the independent evidence suggested the opposite and that the cannula was probably misplaced, the baboons had a smaller

drinking response that was analogous to the control response. Since this procedure is relatively simple to administer, we suggest that it might gain more universal usage for this purpose. One should also, of course, utilize other indices where possible, such as the lack of backflow of CSF when the cannula is inserted, and, more importantly, anatomical confirmation of placement when an experiment (and animal) is terminated.

We also found that the repeated administration of A-II over relatively long injection intervals resulted in a decline of the magnitude of the response although the response was still present (cf. Fig. 2). There is no obvious precedent for this observation in the literature since virtually all other reports in which drinking was observed after IVT A-II were based upon shorter experiments (14 days maximum). There are, however, reports that when A-II has been administered more than once within a several-day interval to rats, the response diminishes or disappears (14). The significance of this alteration of responsiveness to A-II is unclear. It is unlikely that the distribution of A-II within the CSF of the ventricular system can account for the difference since the independent verification of cannula patency was obtained and since the particular cannula used did not seem to be a factor. It also appears unlikely that pharmacological tachyphylaxis to A-II can account for the reduced dipsogenic effect because of the long intervals (up to 11 months) between injections. Finally, it is possible that this alteration of apparent responsiveness may be behavioral or may be related to the prolonged insulin treatments. Further work is required, therefore, to elucidate this phenomenon of decreased effect of intracranial A-II in the baboon.

Summary. Cannulas aimed at the lateral cerebral ventricles were surgically implanted in baboons. A-II (5 μg/animal) or control injections were made on subsequent days and water intake recorded to determine the patency of the cannulas. We found that (a) baboons drink considerable water after the IVT administration of A-II, (b) the response diminishes but still persists with repeated A-II tests spread over a several-month interval, and (c) elevated drinking in response to the administration of A-II is a practical way to confirm the success of ventricular cannulation.

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