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The Effects of Intrahypothalamic Injections of Guanethidine on Catecholamine Fluorescence, Food Intake and Temperature Regulation in the Rat[1]

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ARMSTRONG, S., G. BURNSTOCK, B. EVANS AND G. SINGER. *The effects of intrahypothalamic injections of guanethidine on catecholamine fluorescence, food intake and temperature regulation in the rat.* PHARMAC. BIOCHEM. BEHAV. 1(3) 307–312, 1973.– The effects of chronic injections of two dose levels of guanethidine sulphate into the lateral hypothalamus of the rat on eating, drinking, body temperature and on the levels of catecholamines as shown by fluorescence histochemistry were examined over a period of 12 days. During the guanethidine treatment, food and water intake were reduced, body temperature showed a significant rise, and the animals showed a wide loss of catecholamine fluorescence in the hypothalamic area. After cessation of injections, food and water intake as well as body temperature returned to preinjection base levels. Catecholamine fluorescence also returned during the 12 days after cessation of injections. These results are discussed in terms of current hypotheses concerning the hypothalamic adrenergic involvement in consummatory behavior and temperature regulation.

Guanethidine Eating Drinking Temperature Hypothalamus Catecholamine fluorescence

STUDIES of the rat brain have shown that alpha-adrenergic stimulation of the lateral hypothalamus via chronically implanted cannula results in eating behavior in food satiated rats and that muscarinic stimulation results in drinking behavior in water satiated rats [16]. Cholinergic or beta-adrenergic stimulation results in blocking the eating response in hungry rats, while alpha-adrenergic stimulation blocks the drinking response of thirsty rats [16,28]. Desmethylimipramine, which blocks the neuronal uptake of noradrenaline, has been shown to potentiate the effect of noradrenaline on eating behavior [6, 25, 30]. These studies suggest that alpha-adrenergic transmitters are involved in the regulation of food and water intake.

Short term studies on core temperature in the rat indicate that hypothalamic cholinergic stimulation results in hyperthermia, noradrenergic stimulation in hypothermia [1]; dopaminergic stimulation also leads to hypothermia [22]. Although results from different laboratories are inconsistent [1], the data suggest that catecholamines are involved in temperature regulation.

Acute administration of guanethidine is known to lower or depelete the catecholamine content of peripheral adren-

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ergically innervated organs [3, 15]. Chronic administration results in long-lasting damage specifically to adrenergic nerves which persists even months after cessation of treatment [4, 10, 12, 17, 20]. However, the action of guanethidine on the central nervous system is less clear. This is partly because little of the guanethidine when injected peripherally crosses the blood brain barrier, and partly because few studies of intracranial injected guanethidine have been reported [13, 21, 23].

The present experiments were designed to study the effects of guanethidine injected intrahypothalamically on catecholamine levels and its effect on consummatory behavior and temperature regulation.

METHOD

Animals

Experimental animals consisted of 25 naive, male, Wistar rats, weighing at least 250 g at the time of surgery. Rats were housed in individual cages of wire mesh, in a continuouly illuminated room, maintained at a temperature of 70± 2°F. After surgery, food and water were available ad lib.

Surgery

Two stainless steel cannula [7] were bilaterally implanted at the level of the hypothalamus, by a stereotaxic instrument. The following co-ordinates were used (26): A + 0.8, L ± 1.9, and H − 8.5 (relative to bregma).

Drugs

Guanethidine sulphate (CIBA) was dissolved in distilled water, 32 μg/μl, and made isotonic to cerebro-spinal fluid by addition of NaCl to 0.154 M. 0.154 M NaCl was used as placebo.

Procedure

Following at least a two week postoperative period, rats were placed on a 20 hr food deprivation schedule, water ad lib. Rats were supplied preweighed food consisting of a balanced powdered diet between 11 a.m. and 3 p.m. At the end of the 4-hr eating period, a water reading was taken, food was removed, and all spillage collected and included in the weighings. Rectal temperatures were taken daily at 9:30 a.m., using a SMEC-10 electronic thermometer. This also accustomed rats to daily handling. After three weeks, rats were considered to have adapted to the 4-hr feeding schedule.

Thirteen rats were chosen and randomly assigned to four groups: 1 μl placebo group (N = 2); 1 μl drug group (N = 4); 2 μl placebo group (N = 3); and a 2 μl drug group (N = 4). A further 12 rats were used in a repeat of the experiment in order to gain additional histochemical data for the 2 μl injection dose. All injections were given bilaterally.

In order to minimise cellular damage due to pressure on injecting, injection rate was limited to 1 μl per min. To minimise constraint upon the rat, and thus stress, due to handling [8], the animals were placed in a 12 in. square, clear perspex box during injection. The injection needle plus an 18 in. length of Teflon tubing was attached to an Agla micrometer syringe outfit, which was clamped to a ring stand. Thus the rat was free to move around during injection, and the experimenter was free to manipulate the micrometer syringe. Food and water readings were taken hourly for the 4-hr test period, and, in addition, overnight water readings were kept. Injections were carried out for 12 days; injection treatment then stopped and food and water intake and rectal temperatures were recorded for a further 11 days.

Histochemistry

The fluorescence histochemical method for localizing monoamines was used in accordance with the Falck-Hillarp technique [11]. Rats were guillotined and the brains were quickly removed through the dorsal surface of the skull. The hypothalamus was dissected out [14], frozen in liquid propane cooled with liquid nitrogen and then freeze-dried at −38°C and 10^{-3} mmHg, using P_2O_5 as a moisture trap, for 36 hr. The tissue was then allowed to return to room temperature over 7 hr and heated to 35°C before incubation in a sealed vessel at 80°C for 1½ hr with paraformaldehyde at optimal humidity. After vacuum embedding in paraffin wax, sections (15 μ) were mounted on heated glass slides with paraffin oil and examined in a Leitz Ortholux fluorescence microscope with an optical system as described elsewhere [4]. In this study no attempt was made to distinguish the fluorescence of adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine.

RESULTS

Behavior

The first injection of both dose levels of guanethidine reduced food intake in all rats. This reduction in food intake continued on all 12 subsequent treatment days, although there were some fluctuations. On cessation of drug treatment, rats resumed their preinjection level of food intake within one day. The typical food intake pattern is shown in Fig. 1. Binomial test [29] showed that the reduction in food intake for five out of six rats was significant ($p<0.05$). Further, the probability of rejecting five out of six null hypotheses at $p = 0.05$ is less than 0.0001.

Within the 4-hr feeding period, drug rats showed a different eating pattern as compared to the placebo rats. Placebo treated rats consumed most of their food within the first two hr of the feeding period. The 1 μl group followed this pattern, but at a reduced level of food consumption, while food intake for the 2 μl group was consumed more evenly over the 4 hr.

Water intake for chronically treated rats followed the pattern of food intake, but at a lower level. However, drinking during the night showed an increase, although some individual differences were to be found. The 1 μl drug group continued to drink overnight at an increased level over the 12 injection days; the 2 μl drug group, although at first increasing their drinking overnight, reduced this intake after several days of injection.

Body temperature for chronically treated rats showed a significant rise during drug administration as compared to their preinjection temperatures and the placebo controls. Binomial test [29] showed that this increase was significant ($p<0.05$) for all drug treated rats. However, within two days of cessation of drug injections, temperatures returned to predrug levels.

Fluorescence Histochemistry

Throughout the injection series catecholamine fluores-

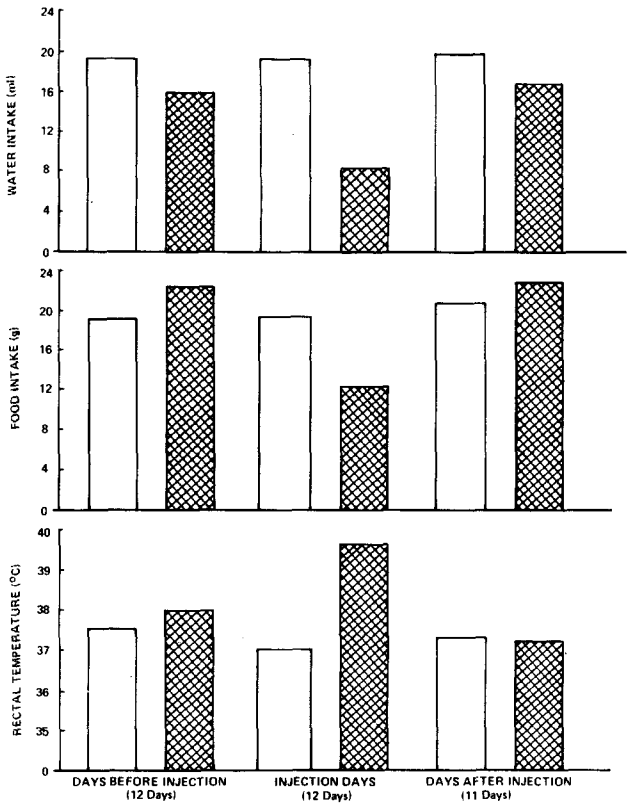

FIG. 1. Medians of drinking, eating and rectal temperature for two animals. Experimental animal (crossed hatched bars) chronically treated with bilateral intrahypothalamic injections of guanethidine sulphate (2 μl, 32 μg/μl [1]. Control animal (open bars) treated with 2 μl isotonic saline intrahypothalamically.

cence of the hypothalami from placebo treated rats was similar to that of the hypothalami from control rats (Fig. 2A). There was often a considerable accumulation of specific fluorescence adjacent to the cannula tract representing a pile-up of catecholamines in the severed axons. Intracranial injections of guanethidine resulted in a widespread depletion of hypothalamic catecholamines as shown by fluorescence histochemistry. After a single 2 μl dose there was a depletion of the majority of normally fluorescent fibres within a 1 mm radius of the cannula. By 3 and 5 days the depletion was much more widespread and after 12 days treatment fluorescent fibres were absent within a radius of 3 mm from the tip of the cannula – including such regions as the paraventricular nucleus and the arcuate nucleus of the hypothalamus, the lateral and anterior hypothalamic areas and the ventromedial nucleus of the hypothalamus. In addition the perivascular adrenergic fibres supplying the ventral cerebral arteries were depleted of noradrenaline along the whole length of the tissue taken (5–7 mm) (see Fig. 2B). No accumulation of specific fluorescence was found adjacent to the cannula during guanethidine treatment.

Some regions were apparently more resistant to the depleting action of guanethidine. The level of fluorescence in the median eminence dropped to about 50% of control levels after 5 days treatment and to about 20% by 12 days. This remaining fluorescence was mainly located on the ventral margin of the median eminence. The supra-optic nucleus and occasionally perivascular fibres around blood vessels within the optic tract were also depleted more slowly so that after 12 days treatment some fluorescence (considerably less than control levels) often remained. Fluorescent cell bodies were also visible adjacent to the fornix (Group A 13, [33],) in the arcuate nucleus (Group A 12, [33],) and in the substantia nigra throughout the guanethidine treatment.

One day after cessation of treatment there were still no fluorescent fibres visible in the region adjacent to the cannula. There was no evidence of accumulation of specific fluorescence along the cannula tract. Occasionally faintly-fluorescent fibres were visible 2–3 mm from the tip of the cannula and there was some return of fluorescence to the perivascular fibres supplying the ventral cranial arteries. Two days after the cessation of treatment there was some return of fluorescence in the region around the cannula, to approximately 20% of normal levels. There was also some evidence of a pile up of fluorescent material in the severed axons alongside the cannula tract. By 7–12 days after cessation of treatment the fluorescence of the hypothalamus was comparable to control levels and there were dense fluorescent accumulations alongside the cannula tracts.

DISCUSSION

Injection of guanethidine sulphate to the lateral hypothalamic area resulted in a reduction of food and water intake and a rise in body temperature. Rats continued to eat and drink at reduced levels throughout the injection period in spite of the fact that histochemical results taken on injection days five and 12 showed that most of the catecholamine containing fibres in the lateral hypothalamus had lost their fluorescence.

The results lend further support to catecholamine involvement in central regulation of eating behavior, but it is interesting to note that the reduction in consummatory behavior was incomplete. In this respect results differ from those of lesion studies in this area [2,31] resulting in adipsia and aphagia. Thus, the present results could suggest that some other brain areas not affected by guanethidine treatment or some other transmitter substance within the hypothalamus are also involved in the regulation of food intake in the absence of hypothalamic adrenergic mechanisms.

Another explanation for the incomplete reduction in consummatory behavior could be that some residual activity in the adrenergic neurons remains despite the widespread loss of fluorescence. This is consistent with studies of the peripheral system where it has been shown that some adrenergic neurons are more resistant to guanethidine [4, 10, 12, 17] and where a small response to adrenergic nerve stimulation remains in vasa deferentia which are devoid of fluorescence [9].

The initial reduction in water intake may be due simply to reduced prandial drinking, i.e. a food deprived rat reduces its drinking and vice versa. The initial compensatory drinking that occurred during the night seems to support this hypothesis. However, long term results may be explicable in terms of an enzyme induction theory [19] i.e. changes in noradrenaline levels in the hypothalamus are accompanied by respective changes in choline acetylase, and thereby inferred changes in acetylcholine. Thus, changes in noradrenaline levels due to drug depletion may

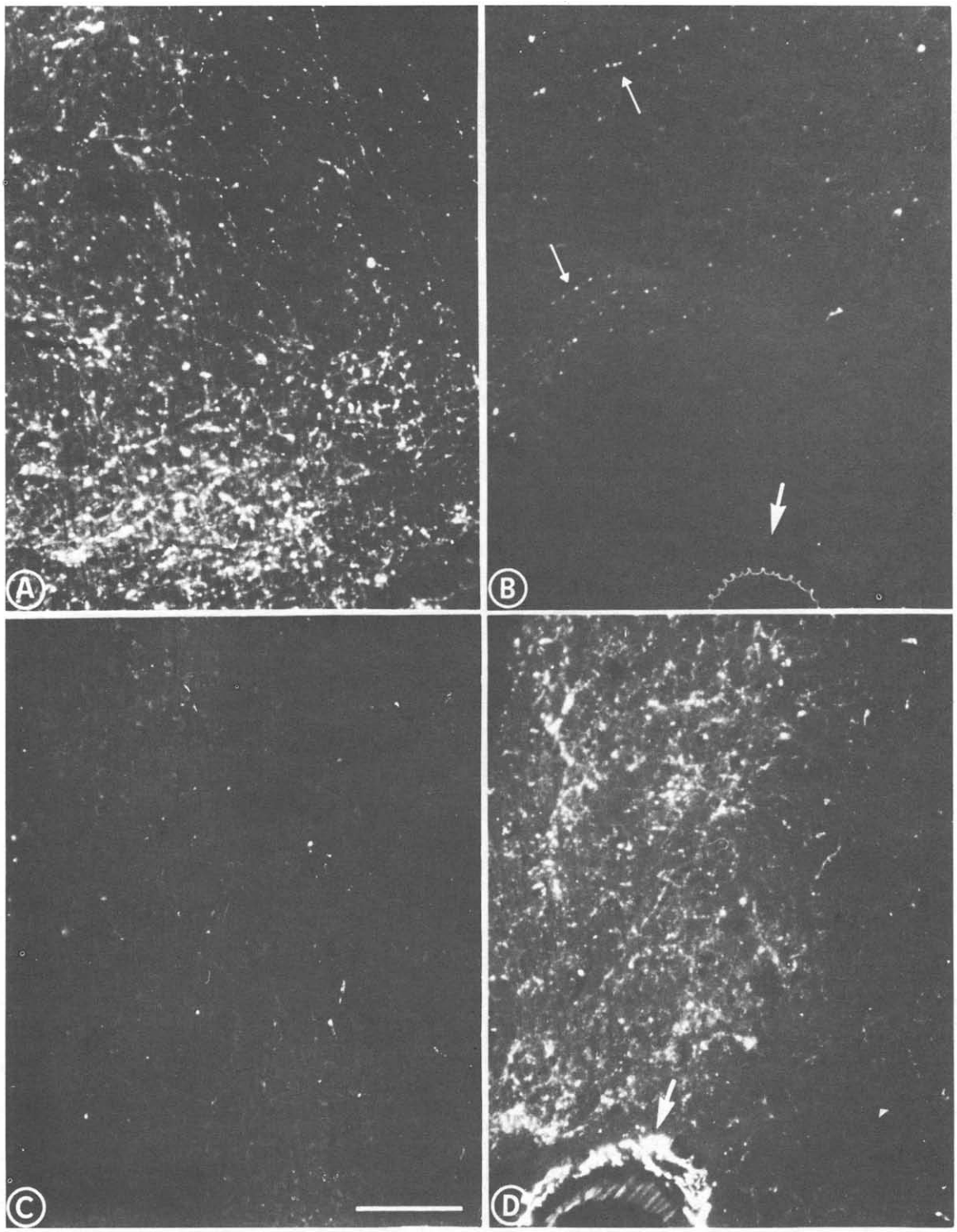

FIG. 2. Fluorescence histochemistry of the lateral hypothalamic area of the rat brain. Rats were cannulated bilaterally in the lateral hypothalamic area and killed after the following treatments. Calibration = 100 μ and applies to all figs. A. Placebo daily for 5 days. B. 2 μl of guanethidine solution (32 μg/μl) daily for 5 days. Note that only a few fluorescent fibres remain (small arrows) and the absence of fluorescent fibres around the ventral cerebral artery (large arrow). C. 2 μl of guanethidine solution (32 μg/μl) daily for 12 days. Note complete absence of fluorescent fibres. D. Animal killed 12 days following 2 μl of guanethidine solution (32 μg/μl) daily for 12 days. Note the return of fluorescence to the lateral hypothalamus and to the perivascular adrenergic network (arrow).

be accompanied by changes in acetylocholine and therefore drinking [18,19]. It is also possible that the depletion of noradrenaline, which normally has an inhibitory effect on behavior effected by cholinergic compounds [28], permits a sudden increase in acetylcholine, which in turn could lead to a cholinergic feedback blockade. This would be a similar result to the reduction in drinking obtained with high doses of carbachol [24,27]. Finally, it is not inconceivable that the deprivation state of the organism has different behavioral effects as a result of catecholamine changes [25].

These points made on the effects of the cholinergic-adrenergic interaction in regard to drinking behavior may also be relevant to hypothalamic temperature regulation in the rat. However, most studies have been concerned with short term drug effects, rather than the biochemical involvement of long term temperature control. The rise in body temperature may be due to a general drug effect, but this is unlikely as previous reports have indicated that a reduction in food intake results in an eventual drop in body temperature [32], and in this study the rise in temperature was consistent and reliable.

The histochemical results provide direct evidence of the depleting effect of guanethidine on catecholamines in the central nervous system. They support the findings from ventricular injections [13,23], and clarify the conflicting evidence which has resulted from studies using peripheral injections of guanethidine [3,15]. The greater resistance of the ventral border of the median eminence, Groups A12 and A13 neurons [33] and substantia nigral neurons to the depleting action of guanethidine suggests a differential specificity for noradrenergic neurons as compared to dopaminergic neurons. This possibility has been the basis of further investigations which will be published in a separate communication.

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