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BRIEF COMMUNICATION

Comparison of the Effect of Prostaglandin E_1 and Norepinephrine Injected into the Brain on Ingestive Behavior in the Rat

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WHISHAW, I. Q. AND W. L. VEALE. *Comparison of the effect of prostaglandin E_1 and norepinephrine injected into the brain on ingestive behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(3) 421–425, 1974. – Norepinephrine hydrochloride (NE) in doses of either 5 or 25 μg and prostaglandin E_1 (PGE_1) in doses of either 5 or 50 ng were made directly into the substance of the hypothalamus as well as other limbic structures of the unrestrained, unanesthetized rat. The animals had free access to food and water at all times. NE injected into the perifornical area of the hypothalamus, the hippocampus and the amygdala elicited vigorous eating. PGE_1 did not produce eating which differed from that observed following control injections of a physiological control solution. Drinking was not observed following injections of either NE or PGE_1 at the dose used. These data indicate that PGE_1 does not play a direct role in the hypothalamic and limbic control of ingestive behavior.

Norepinephrine induced eating Chemical stimulation Prostrglandin E_1 Ingestive behavior

MICROINJECTIONS of norepinephrine (NE), when applied directly to certain regions of the substance of the brain either in solution or as crystals produces eating in the rat [3, 4, 9, 10, 15]. This work has been consistent in identifying the perifornical area of the hypothalamus as the area from which the best eating response can be produced with the lowest doses of NE, however, Booth [3] has provided evidence that NE injected at many different loci along the stria medullaris may also elicit feeding. In addition, eating has also been elicited or ongoing feeding responses facilitated, following injections of NE into the cerebral ventricular fluid spaces [12], the hippocampus [5] and the amygdala [9]. Recently, it has been shown that prostaglandin E_1 (PGE_1) may also be involved in the production of hyperthermia in several different species including the rat, cat and rabbit [1, 6, 11, 16, 17] and although the evidence is not convincing, it has been suggested that PGE_1 may also be involved in the central nervous system's control of feeding [1, 2, 14].

The present experiments were carried out to examine further the influence of PGE_1 injected directly into the brain on eating and drinking. Norepinephrine was injected as well so that the loci in the brain in which the application of NE would produce eating could be identified and the feeding responses obtained compared to those observed by other workers. NE and PGE_1 were injected into hypothalamic and other regions of the limbic systems and observations were made on post-injection eating and drinking.

METHOD

Animals

The experiments were performed with 40 male, albino Sprague-Dawley rats ranging in weight from 300–350 g. The rats were housed individually in constant light with free access to food and water throughout the experiments.

Surgical Procedure

Each rat was anesthetized with pentobarbitone sodium

injected intraperitoneally (40 mg/kg). Using standard stereotaxic procedures, two guide tabes were implanted bilaterally so that the tip of each was positioned 3 mm above the site of injection. Each guide tube was of 20 g stainless steel tubing and fitted with an indwelling stylet of corresponding length also made of stainless steel tubing. The guide tubes were fixed into the skull with cranioplast cement and stainless steel anchor screws. Post-operatively, penicillin was given for 3 days. Seven to ten days elapsed before experiments were begun.

Injection Placements

Sites selected for injection were based on the rat stereotaxic atlas of Pellegrino and Cushman [13]. Co-ordinates for the injection sites were: anterior hypothalamus, anterior to bregma 2.0–2.4 mm, lateral to midline 1.5 mm and ventral from the dura 8.2 mm; lateral hypothalamus, 0.6 to −1.6 mm anterior, 1.5 mm lateral, 9 mm ventral; posterior hypothalamus, −2.0 to −3.0 mm anterior, 1.5 mm lateral and 7.5 to 8.2 mm ventral; hippocampus, −2 and −3.2 mm anterior, 2 and 5 mm lateral, 3.5 and 8.5 mm ventral (3 rats received placements in the dorsal and 3 in the ventral hippocampus); amygdala, −1.0 mm anterior, 5 mm lateral and 9 mm ventral. Coordinates for the perifornical injection site were the same as those described by Booth [4].

Procedure

Microinjections were made using 27 g hypodermic tubing which was lowered through the guide tube so that its tip extended beyond the guide tube to the desired depth into the tissue to be stimulated. The injection cannula was connected to a length of PE 20 tubing, both were filled with and stored continuously in 70 per cent ethyl alcohol solution which was then washed out thoroughly with sterile, pyrogen free saline before being filled with the solution to be injected. The PE tubing was attached to a 10 μl syringe mounted on an infusion pump. Injections of a volume of 0.5 μl were given over a 30 sec interval.

Both NE and PGE_1 were dissolved in a modified Krebs solution which was made from ion exchanged, glass-distilled water which was passed through a sterilized millipore filter (0.22 μ), and which contained: Na 143.0 mM; K 5.8 mM; Cl 128.1 mM; glucose 5.6 mM; Mg 1.2 mM; SO_4 1.2 mM; H_2PO_4 1.2 mM; HCO_3 25.0 mM. This solution was shown to be pyrogen-free by independent assay. The animals received injections of modified Krebs solution, 5 or 50 ng of PGE_1 or 5 or 25 μg NE at each injection site. Any one animal received a particular dose only once. The animals were brought to the experimental room at least one hour prior to each injection. Each rat received two Purina rat chow food pellets which were weighed at the beginning of the adaption period and again at 15 min intervals until the experiment was complete but in every case for at least 1 hr following the injection. Crumbs from chewing were caught on a paper and were also weighed. The test cage was of wire mesh and measured 9.5 × 7.5 × 8.0 in. Throughout the tests, calibrated water bottles were present for the duration of each experiment and water intakes were recorded at 15 min intervals. Injections were given in a randomized order and a period of at least 48 hr elapsed between successive microinjections to any one animal.

Histological Examination

At the conclusion of the experiments, the position of the cannulae tips were verified using standard histological techniques. A dye was injected into the injection loci and the animals were anesthetized with pentobarbitone sodium. They were then perfused through the heart, first with a saline solution and then with a solution of 10 per cent Formalin which contained 0.9 per cent NaCl. Frozen sections were cut at 30 μ and then mounted and stained with thionin.

RESULTS

Both NE and PGE_1 were injected in more than 80 sites within the brain of the unanesthetized rat. Eating which

TABLE 1

THE EFFECTS OF PGE_1 IN DOSES OF 5 ng (L) AND 50 ng (H) AND OF NE IN 5 μg (L) AND 50 μg (H) ON FEEDING IN THE RAT. MEAN (gm) AND STANDARD DEVIATION FOR FOOD CONSUMPTION DURING THE FIRST HR FOLLOWING MICROINJECTION. THE NUMBER OF ANIMALS INVOLVED (n) IS GIVEN. THE DASH (–) INDICATES THAT NO EATING WAS OBSERVED.

	AH	LH	PH	PFA	AMY	HIP
Control	–	–	–	0.1 ± 0.5	0.1 ± 0.02	–
(L) PGE_1	–	–	–	0.3 ± 0.8	0.1 ± 0.03	–
(H) PGE_1	0.3 ± 1.2	0.5 ± 0.9	–	0.3 ± 0.7	0.6 ± 1.1	–
(L) NA	0.1 ± 0.8	–	1.2 ± 1.8	3.6 ± 4.9*	2.2 ± 0.9*	4.2 ± 0.8*
(H) NA	–	1.2 ± 1.1	–	0.2 ± 0.4	4.7 ± 0.7*	5.5 ± 0.6*
n	6	6	6	10	6	6

*$p>0.001$ difference from control values

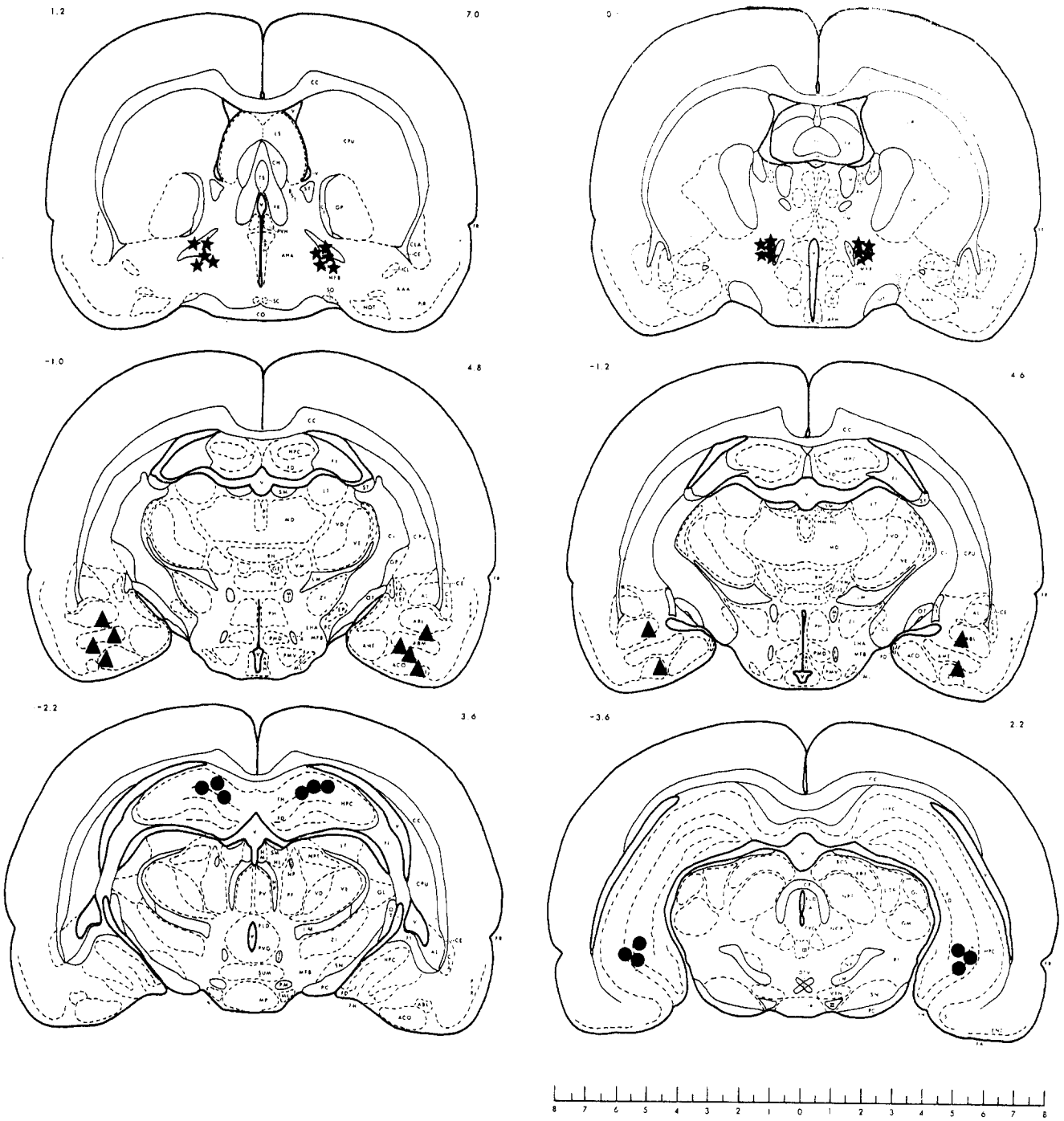

FIG. 1. Location of placements of cannula tips in the hypothalamus (top) amygdala (middle) and hippocampus (bottom) showing sites from which eating was elicited with injections NE.

differed significantly from control levels occurred following injections of NE into the perifornical area, the amygdala and the hippocampus. As can be seen from Table 1, the mean and standard deviation of food intake during the first hour following the injection of 5 μg NE into the perifornical area was 3.6 ± 4.9 g, whereas that following the injection of 25 μg NE was 0.2 ± 0.4 g. In both the amygdala and hippocampus there was a dose dependent eating response following injections of NE. The 5 μg dose in the amygdala producing a mean intake of 2.2 ± 0.9 g and the 25 μg dose producing 4.7 ± 0.7 g. In the hippocampus the 5 μg dose produced a mean value of food intake of 4.2 ± 0.8 g whereas the higher (25 μg) dose produced a mean of 5.5 ± 0.6 g. The food consumption by rats with either dorsal or ventral hippocampal placements was similar following injection and this data was pooled. It can be seen in the table that some

eating was observed following the injection of 5.0 μg NE into the region of the posterior hypothalamus was well as when 25 μg NE was injected into the lateral hypothalamic area. These values do not differ statistically from baseline intakes.

No significant increases in food intake were seen following the injection of PGE_1 into the sites tested. These included the anterior hypothalamic area, the lateral hypothalamic area, the perifornical area and the amygdala. As can be seen from Table 1, slight eating was obtained in some of these loci, however, the amount of food ingested was not statistically significant from that ingested following control injections with a modified Krebs solution. Drinking was not observed at any time following the injection of either NE or PGE_1 in any of the loci tested.

Figure 1 is a summary of the histological findings indicating the sites into which injections of NE produced eating.

DISCUSSION

The finding that of NE injected into the hypothalamus, particularly the perifornical area, produces eating confirms earlier work [4, 7, 9]. Similarly, it has also been observed previously that lower doses of NE are more effective in eliciting eating from the hypothalamus than higher doses [10]. Coury [5] demonstrated that eating was produced following the injection of NE into the hippocampus. Grossman [9] found enhanced eating in deprived rats but only slight eating in sated rats following injections of NE into the amygdala. There is in some of this work the possibility that because of the close proximity of limbic sites of injection to the cerebral ventricular spaces there may be diffusion of the drug into the ventricles, an area from which vigorous eating has been obtained [12]. This possibility is remote in the work reported here since injection of bromophenol blue into the same loci was not observed to have made its way into the ventricular system. Alternately, the drug may cause electrographic seizures in the limbic structures and thus produce eating in a manner similar to that following electrical stimulation of the septum [19]. The most likely explanation is that the structures into which NE was injected and eating was produced may contain, as Booth [4] and Grossman [8,9] have suggested, neurons involved in the regulation of ingestive behaviour which have their activity modulated to some degree at least by NE.

It is clear from the results of the present experiments that PGE_1 does not produce eating or drinking when it is injected into sites from which eating can be elicited following the direct injection of NE. These results are compatible with the work of Baile and his colleagues [1,14] in the rat as well as the results which indicate that PGE_1 does not elicit eating or drinking in the cat or rabbit [17]. Some evidence has been obtained for the concept that PGE_1 may inhibit feeding when injected into some loci [1], however, the amount of PGE_1 injected by these investigators to produce the effect was much greater than that used in this work. It has been shown clearly that PGE_1 is a potent hyperthermic agent in the rat [17]. The inhibitory effects on eating shown in earlier work [1] may have been due to some degree by temperature increases resulting from the injection of PGE_1. The amounts of PGE_1 used in the present work have been shown to be particularly potent in eliciting fevers, in fact, when 5 or 50 ng PGE_1 is injected into the anterior hypothalamus temperature increase of 2–3° C within 30 min were produced [17,18]. We selected the doses of PGE_1 to be consistent with those known to produce temperature increases when applied directly to the anterior hypothalamus. In some instances a small amount of eating did occur following injections of PGE_1 in some sites; however, there was no consistent relation between any loci and this sporadic eating. In addition, there was no indication of a dose dependent response and the amount eaten was consistently less than that obtained with NE. Similar sporadic eating was observed following injections of the modified Krebs solution which was used as the injection vehicle. Our results provide evidence that PGE_1 is not directly involved in the central nervous system's regulation of ingestive behavior.

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