EFFECTS ON FEEDING BEHAVIOUR AFTER INTRAHYPOTHALAMIC INJECTIONS OF 6-HYDROXY-DOPAMINE IN RATS

JOSEPH L. SLANGEN
Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands

DIRECT application of norepinephrine, epinephrine and clonidine to the perifornical region of the rat hypothalamus induces eating in food satiated rats (BOOTH, 1967; SLANGEN and MILLER, 1969; BROEKKAMP and VAN ROSSUM, 1972). This effect is prevented by pretreatment with alpha adrenergic receptor blocking agents and is not antagonised by the beta adrenergic blocker propranolol (BOOTH, 1968; SLANGEN and MILLER, 1969). The norepinephrine induced eating is potentiated by prior intrahypothalamic administration of desmethylimipramine (BOOTH, 1968; SLANGEN and MILLER, 1969). Eating can also be elicited by intrahypothalamic application of a monoamine oxidase inhibitor followed by a catecholamine depleting agent (SLANGEN and MILLER, 1969). The feeding response can not be induced however by beta-adrenergic agonists, dopamine or serotonin (BOOTH, 1968; SLANGEN and MILLER, 1969). The site where noradrenaline is most effective in eliciting eating is the mediolateral hypothalamus near the fornix approximately 1·3 mm from the midline (BOOTH, 1967). Ungerstedt has reported that bilateral destruction of the nigrostriatal dopamine system is associated with a syndrome of adipsia and afagia. In particular the bilateral injections of 6–8 μg of 6-hydroxy-dopamine (6-OHDA) in the dopamine axons assembled in the lateral hypothalamus resulted in prolonged afagia and adipsia. Afagia and adipsia also occur after interruption of the nigrostriatal system outside the hypothalamus (UNGERSTEDT, 1971a). Obviously this effect cannot be attributed to the destruction of the postulated "feeding system" of noradrenergic fibres in the hypothalamus. In the lateral hypothalamus the dopaminergic nigro-striatal system passes very closely to the perifornical area in which noradrenergic neurons are assumed to mediate the eating response (UNGERSTEDT, 1971a). It is not unlikely that the results obtained with chemical stimulation of the perifornical area are dependent upon interac with the nigro-striatal system and therefore the question to be resolved is whether an intrahypothalamic adrenergic feeding system can be distinguished from the dopaminergic nigro-striatal system. In all experiments male albino rats (200–350 g) of an inbred Wistar strain were used. Under anaesthesia the animals were placed in a stereotaxic instrument and received bilaterally an injection of 8 μg 6-OHDA and 0·8 μg ascorbic acid dissolved in 1 μl of distilled water. Control rats received 0·8 μg ascorbic acid in 1 μl of distilled water only. The injections were aimed at an A–P level of 4620 according to the atlas of König and Klippel and at different lateral positions ranging from 0·4 to 3·0 mm from the midline. Up to 1·4 mm lateral the depth of the injection site was kept constant at 3 mm below horizontal zero. From 1·4 mm lateral onwards the depth was decreased in order to keep the injection sites parallel to the optic tract. Body weight and food and water intake were recorded

for two weeks. Animals were assigned to 10 different groups depending on the site of injection. The only animals included in this report are those in which the location of the injection site on the left and right sides of the brain corresponded exactly.

Afagia occurred only when 6-OHDA was injected in an area 1·6 to 2·1 mm from the midline. Ungerstedt has localised the axons of the nigro-striatal system to this area (UNGERSTEDT, 1971a). Injecting 6-OHDA into the more medially located perifornical area does not result in any impairment of feeding behaviour. If a noradrenergic feeding system had existed in this area an impairment would have been expected after 6-OHDA. The lack of any effect may be attributed to an uncomplete destruction of noradrenergic terminals. We therefore repeated the experiment in a series of points between 0·4 and 1·6 mm lateral to the midline. On each side of the brain two injections were given into the hypothalamus but at a vertical distance of 1 mm from each other. After histological examination animals were assigned to 6 different groups depending on the sites of injections. Afagia and adipsia were observed only in the group which received injections 1·6 mm lateral from the midline. Injections made in the area extending from 0·4 to 1·4 mm laterally caused a transient loss of body weight lasting for a few days only.

In order to ascertain that 6-OHDA reached the exact site from which noradrenergic eating could be elicited cannulas were implanted bilaterally in the perifornical region of 30 rats. Per cannula rats were tested three times. Injections were given every other day. In 13 rats the food intake after injection of 30 nmol norepinephrine (NE) was 2·0 $\pm$ 1·1 g (mean $\pm$ S.D.). Seven rats were weak eaters. Their mean eating response to 30 nmol NE was 1·1 $\pm$ 0·7 g. After the NE-test 8 μg 6-OHDA was injected bilaterally. It was found that 6-OHDA still was without an effect on eating in these 20 rats. When 30 nmol NE was injected 7 days after the 6-OHDA treatment an enhanced feeding response was obtained in two tests per cannula. The mean food intake of the strong eaters was 3·1 $\pm$ 1·3 g and of the weak eaters 3·2 $\pm$ 1·4 g. The difference between the mean response to NE before and after the 6-OHDA injection is statistically significant in both groups at the 0·1 % level (two tailed Student's t-test). This enhanced eating response to NE suggests that some change in the noradrenergic terminals had occurred as a result of the 6-OHDA treatment. In the same animals a second intrahypothalamic injection with 16 μg 6-OHDA was given. Although this treatment must have caused an even greater presynaptic degeneration no impairment of feeding was observed in 14 days.

In another series of experiments cannulas were implanted bilaterally in the nigro-striatal system of the lateral hypothalamus. 24 animals were tested three times per cannula with saline, 30 nmol NE and 30 nmol dopamine (DA) in a simple crossover design. Results are reported for 15 rats in which the placements of the cannula tips were found at the point where lateral hypothalamus and capsula interna meet. The response to NE (0·8 $\pm$ 0·6 g) and to DA (0·7 $\pm$ 0·6 g) was not different from the control response to saline (0·6 $\pm$ 0·4 g). The bilateral administration of 6-OHDA (8 μg) in these animals did not result however in any feeding deficits. In a new attempt to stimulate the nigro-striatal system cannulas were implanted bilaterally in 15 other rats. In 10 rats histological verification showed that both cannulas had penetrated at a reasonably correct depth the lateral hypothalamic region extending from 1·8 to 2·2 mm lateral. Each implant was tested three times with 30 nmol NE and saline. No eating responses were seen. After the bilateral administration of 8 μg 6-OHDA

no changes in consummatory behaviour were observed. The 6-OHDA treatment was repeated after 7 days and again no effects on feeding were obtained. Finally these animals were tested with 30 nmol NE again but no eating response was found that differed from the response to NE before the 6-OHDA treatment. The fact that 6-OHDA caused no afagia when given via cannulas that were aimed at the nigro-striatal system may be caused by technical difficulties. It is our feeling that with the cannula technique the site of the nigro-striatal system is more difficult to localise than with the single injection technique. The reported results support entirely the concept of an anatomical and functional distinction between the hypothalamic nigro-striatal DA system and the perifornical NE system (UNGERSTEDT, 1971b). NE can elicit eating only in the perifornical region and has no such effect in the directly adjacent nigro-striatal DA system. A single injection of 8 μg 6-OHDA causes afagia and adipsia when administered in an area of about half a millimeter width in which the nigro-striatal DA system has been localised and has no such effect when administered in the more medial lateral hypothalamus in which the NE "eating" system has been localised. After administration of 6-OHDA in the NE system a supersensitivity to NE stimulation has been observed whereas no such effect has been seen in the DA system. Since we have not analysed in sufficient detail the food intake of our rats we may have overlooked small effects of 6-OHDA injected into the perifornical region. But the lack of any great effect on the regulation of food intake after degeneration of the perifornical NE system suggests that this system is not a major control system for food intake. It may be however that the antagonistic relationship as proposed by LEIBOWITZ (1970) and GOLDMAN *et al.* (1971) between an alpha adrenergic "feeding" system and a beta adrenergic "satiety" system, both localised in the perifornical area, is such a completely complementary one that the observed ineffectiveness of 6-OHDA may have been due to an equivalent catecholamine depletion in the two opposing systems. This hypothesis predicts the dominance of one system over the other when these systems are not destructed to the same extent.

Acknowledgement—This work was supported by the Foundation for Medical Research FUNGO. I am indebted to Mrs. Marya Hein-Jongepier and Mr. Kees Broekman for their skillfull technical assistance.

REFERENCES

BOOTH D. A. (1967) *Science* **158**, 515–517.
BOOTH D. A. (1968) *J. Pharmacol. Exp. Ther.* **160**, 336–348.
BROEKKAMP C. and VAN ROSSUM J. M. (1972) *Psychopharmacologia (Berl.)* **25**, 162–168.
GOLDMAN W., LEHR D. and FRIEDMAN E. (1971) *Nature, Lond.* **231**, 453–455.
LEIBOWITZ S. F. (1970) *Nature, Lond.* **226**, 963–964.
SLANGEN J. L. and MILLER N. E. (1969) *Physiol. Behavior* **4**, 543–552
UNGERSTEDT U. (1971a) *Acta Physiol. scand. Suppl.* **367**, 1–48.
UNGERSTEDT U. (1971b) *Acta physiol. scand. Suppl.* **367**, 95–122.