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Clonidine Induced Intrahypothalamic Stimulation of Eating in Rats

C. BROEKKAMP and J. M. VAN ROSSUM

Department of Pharmacology, Catholic University Medical School
Nijmegen, The Netherlands

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Abstract. Intracerebral injection of 1 μ l volumes of solutions containing noradrenaline, clonidine, oxymetazoline and phentolamine were performed in the antero-lateral hypothalamus of the Wistar rat at the level of the pars infracommissuralis of the Stria terminalis.

Intrahypothalamic clonidine in a dose as low as 1 μ g strongly increased food intake in satiated rats.

Clonidine (4 μ g) was more potent than noradrenaline (12 μ g) but as potent as oxymetazoline (1.5 μ g).

The clonidine induced eating response was completely blocked by the α -sympatholytic drug phentolamine.

The results emphasize the role of clonidine as an activator of noradrenaline receptors in the central nervous system.

Key words: Noradrenaline — Clonidine — Adrenergic Imidazolines — Central Noradrenaline Receptors — Intracerebral Injections — Hypothalamic Eating System.

Introduction

There is considerable evidence that the adrenergic imidazolines such as naphazoline, oxymetazoline and xylometazoline are direct α -receptor activators (Mujic and van Rossum, 1965). The recent antihypertensive agent, clonidine, an analogue of the adrenergic stimulant imidazolines, likewise shows peripheral α -sympathomimetic effects (Boissier *et al.*, 1968; Kobinger, 1967).

Several reports provide evidence for a central mechanism of action of clonidine (Shaw *et al.*, 1971; Schmidt *et al.*, 1969; Sherman *et al.*, 1968) and α -adrenergic blocking agents inhibit these actions of clonidine (Schmidt *et al.*, 1970 and 1971). However, clonidine has not yet been administered directly into the central nervous system.

Adrenergic stimulants increase eating in satiated animals when introduced directly in the lateral hypothalamus (Grossman, 1962). By using selective α - and β -adrenergic stimulants as well as selective antagonists it was shown that noradrenergic receptor activation is

the cause of intrahypothalamic noradrenaline facilitated eating (Slangen and Miller, 1969).

Since chemical stimulation of eating in the lateral hypothalamus is a clean model for central α -adrenergic drug-receptor interaction, we used this model to study the central effects of clonidine and oxymetazoline.

Methods and Materials

Stereotaxic Operations

Cannulae with an outer diameter of 0.8 mm were implanted under stereotaxic conditions in the antero-lateral hypothalamus of male rats (250–300 g) under pentothal (50 mg/kg) anaesthesia. The tip of the cannula was placed in a position anterior 7.6, lateral 1.4 and deep –2.4, according to the atlas of König and Klippel (1963). The cannulae were fixed to the skull with dental cement, and a circular wall of cement was applied to the extending part of the cannula. The ring so formed was filled with latex (Rhodorsil®) in order to fixate the plug of the cannula, and prevent the rat from scratching the cannula after recovery from surgery.

Intrahypothalamic Injection

The chemicals were dissolved in saline. The freshly prepared solutions were injected with a Hamilton syringe with an adjustable screw on the needle such that the solution could be deposited some distance (e.g. 0.5 mm) beyond the tip of the cannula. By this procedure local destruction of brain tissue could be minimized. 8 consecutive injections were made in the same rat over a period of 3 weeks (see Table 1). The site of injection was controlled at the end of the experiment (Fig. 1). In six out of seven rats, the position of the cannula was correct.

The actual site of injection was established histologically. The average position of the injection site corresponded to anterior 6.25, lateral 1.2 and depth –2.0. This implied that the chemicals were deposited around the pars infracommissuralis of the Stria terminalis.

Chemicals

(–)Noradrenaline (40 nmol = 12.7 μ g of hydrogen tartrate) was used as a reference drug. Clonidine was used initially in a dose of 200 nmol (= 53.3 μ g of HCl salt) but later reduced to 50 nmol (= 13.3 μ g); 15 nmol (= 4 μ g) and 3.84 nmol (= 1 μ g). Oxymetazoline was used in a dose of 5 nmol (= 1.5 μ g). The α -sympatholytic

Table 1. Eating response in grams of rat food consumed during the first hour after the injection in 6 rats following application of saline, noradrenaline, clonidine and oxymetazoline as well as both phenolamine and clonidine in the hypothalamus

Condition or treatment	Nr. 6	Nr. 7	Nr. 8	Nr. 9	Nr. 10	Nr. 11	Mean	SEM
During 1/2-1 h after 24 h food deprivation	8.68 (1 h)	4.30 (1 h)	3.33 (2/4 h)	4.14 (2/4 h)	3.32 (1/2 h)	2.53 (1/2 h)		
Noradrenaline 40 nmol	3.77 (a)	5.26 (a)	3.59 (a)	3.78 (a)	2.81 (a)	3.89 (a)	3.85	± 0.32
Saline	2.25 (b)	0.60 (c)	0.25 (c)	0.61 (b)	0.56 (b)	0.36 (c)	0.77	± 0.30
Clonidine 200 nmol		4.04 (b)	3.31 (b)			6.14 (b)	4.46	± 0.85
Clonidine 50 nmol	2.55 (c)			3.52 (c)	4.33 (c)		3.33	± 0.51
Clonidine 15 nmol	6.86 (d)	6.14 (d)	4.31 (g)	3.56 (g)	2.48 (g)	2.16 (g)	4.25	± 0.78
Clonidine 3.84 nmol	2.56 (g)	2.41 (g)	2.57 (d)	5.00 (d)	5.38 (d)	3.24 (d)	3.53	± 0.54
Phenolamine 53.4 nmol 1/2 h prior to clonidine 15 nmol	1.71 (e)	0.58 (e)	0.19 (e)	1.99 (e)	0.06 (e)	0.13 (e)	0.78	± 0.35
Saline	1.54 (f)	0.13 (f)	1.25 (f)	1.34 (f)	1.24 (f)	0.27 (f)	0.97	± 0.25
Oxymetazoline 5 nmol	2.70 (h)	5.10 (h)	6.56 (h)	6.05 (h)	1.31 (h)	5.51 (h)	4.54	± 0.85

The letter between brackets indicates the sequence of the injections and the date of the experiments. Deprivation test on 24. 7. 1971
a = 27. 7; b = 30. 7; c = 2. 8; d = 5. 8; e = 9. 8; f = 11. 8; g = 13. 8; h = 19. 8.

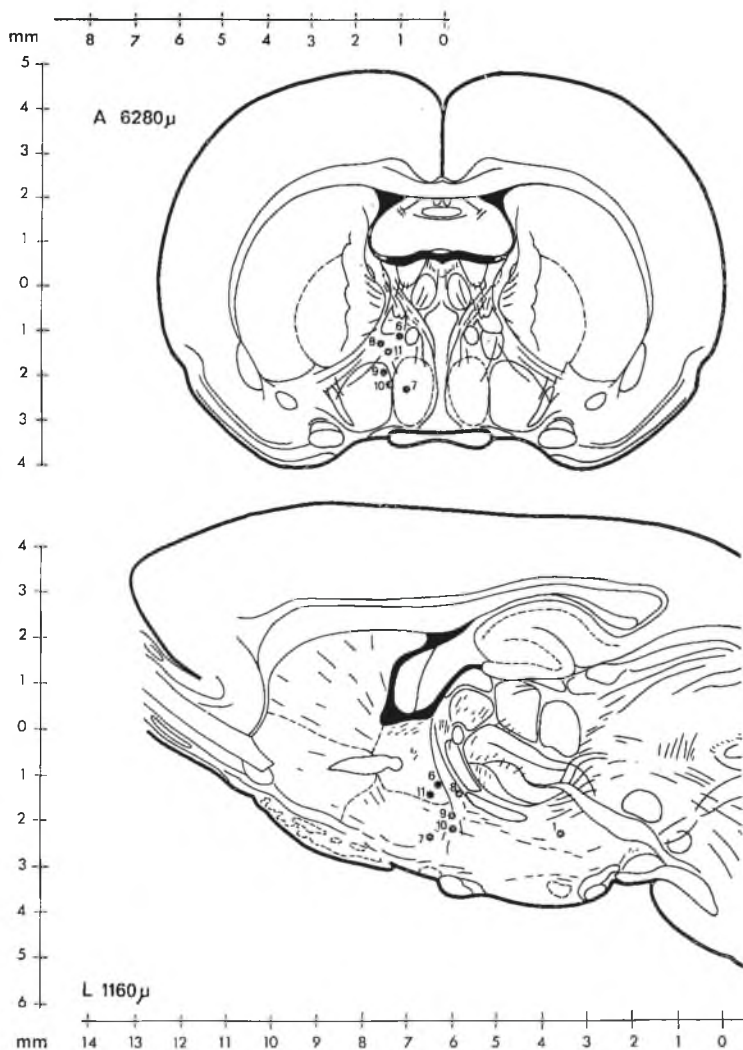


Fig. 1. a Frontal section of the rat brain according to the atlas of König and Klippel in which the sites of application of the chemicals have been indicated. The numbers refer to the different rats. b Sagittal section of the rat brain in which the sites of injection have been indicated. In all rats except number 1, the position of the cannula appeared to be correct. For results of the various experiments see Table 1

drug phentolamine was used in a dose of 53 nmol (= 15 μg) one half hour prior to 15 nmol clonidine. All doses or combinations were applied 6 times except for the initial experiments.

Eating Response

The experiments were carried out in normal rat cages, by measuring the food intake in the first hour after intrahypothalamic injection. The rats were habituated to the cage by placing them in the test cage 5 times during one hour periods prior to the experiments, the last time after 24 h of food deprivation. During the experiments the rats had free access to food.

Results

From the 24 h food deprivation test it appeared that the rats were accustomed to the test cage since they consumed about 3 g in half an hour (Table 1).

The effects of the various intrahypothalamic injections in the individual rats are given in Table 1, together with the sequence of injections in each rat. Injection of the reference substance levonoradrenaline was effective in six of seven rats, causing an average food intake of 3.8 g, whereas the food consumption following a saline injection amounted to about 0.8 g (Table 1). In one rat noradrenaline only induced a food consumption of 1.5 g. Injections deeper in the hypothalamus were no more effective so that this rat was excluded

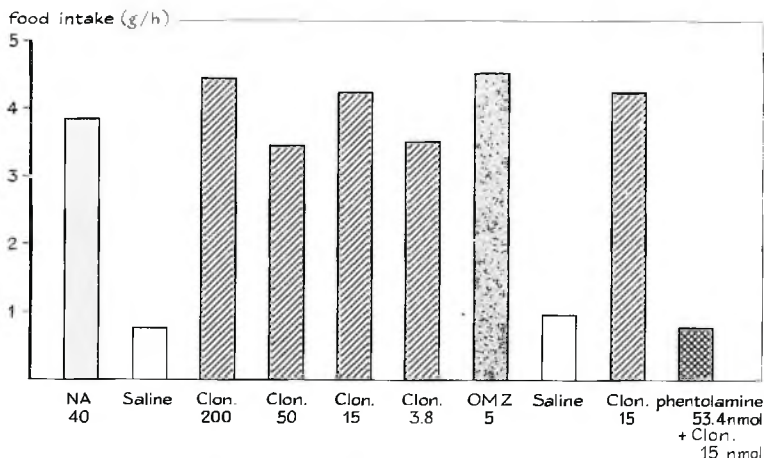


Fig. 2. Mean food intake in 6 satiated rats with cannulae in the correct position following intrahypothalamic injection of saline, noradrenaline, oxymetazoline and clonidine. The alphasymphathomimetic drugs elicit a strong increase in food intake, while the alphasymphatholytic phentolamine completely abolishes the effect of an adequate dose of clonidine

from further experiments. From the histological control it appeared that in this rat the site of injection was too far posterior (Fig. 1).

Clonidine induced a strong eating response in all doses from 200–15 nmol (Fig. 2), and even 4 nmol was fully effective. It may be concluded that intra-hypothalamically injected clonidine is more potent than noradrenaline, since 5 nmol of the latter given under similar conditions is ineffective (Slangen, 1970).

The adrenergic imidazoline, oxymetazoline also strongly increased eating in a dose of 5 nmol, and it is therefore likewise more potent than noradrenaline.

Intra-hypothalamically stimulated eating by clonidine is completely blocked by the α -adrenergic blocking agent phentolamine, when injected at the same site in the hypothalamus, half an hour prior to clonidine. It may therefore be concluded that clonidine acts by activation of noradrenaline receptors in the hypothalamus.

The sites of injection cluster around the pars infracommisuralis of the Stria terminalis. See Fig. 1.

Discussion

During the one hour period following intra-hypothalamic injection, clonidine induces a greater food consumption than noradrenaline. This may however be due to a greater stability of clonidine over noradrenaline, rather than to a greater intrinsic potency. Clonidine differs from oxymetazoline only with respect to its high lipophilicity. This explains why clonidine easily passes the blood-brain-barrier whereas oxymetazoline does not reach the brain in adequate amounts following systemic injection.

Clonidine also exerts local anaesthetic properties equal to procaine (Hoefke and Kobinger, 1966). Clonidine induced eating cannot, however, be caused by a local anaesthetic effect since a selective α -sympatholytic drug phentolamine abolishes the eating response of clonidine.

The imidazolines are pure alpha-receptor activators as they lack beta-sympathomimetic activity (Mujic and van Rossum, 1965).

From studies on specific dopamine- and 5-hydroxytryptamine-sensitive neurons of the snail brain, it is found that clonidine does not activate dopamine- or 5-hydroxytryptamine-receptors (Woodruff, unpublished data).

It may be concluded that clonidine acts on the hypothalamic eating system by activation of noradrenaline receptors which are analogous to the alpha-adrenergic receptors of the peripheral autonomic nervous system.

Cardiovascular studies in our laboratory with intracerebral injection of clonidine provide evidence for a depressor effect of clonidine when injected in distinct areas of the hypothalamus (Struyker Boudier *et al.*, 1972). These studies further substantiate the role of clonidine as an activator of brain noradrenaline receptors.

Following oral administration in patients with hypertension clonidine mainly exerts hypotensive effects. It may however be anticipated that in higher doses various behavioral effects might occur since noradrenaline plays an important role in various parts of the brain.

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C. Broekkamp
Department of Pharmacology
Catholic University Medical School
Nijmegen, The Netherlands