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Hypherphagia caused by muscimol injection in the nucleus raphe dorsalis of rats: its control by 5-hydroxytryptamine in the nucleus accumbens

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Muscimol injection in the nucleus raphe dorsalis caused intense eating by rats with access to food. A dose-related reduction of muscimol's effect was found after bilateral injections of 5-hydroxytryptamine (5-HT) in the nucleus accumbens (dose range 2·2–8·8 μg in 2 μl) but no effect was observed when an even higher dose (17·6 μg) of 5-HT was injected in the caudate putamen. Eating by food-deprived rats was not changed by any dose of 5-HT injected either into the nucleus accumbens or the caudate putamen. (+)-Norfenfluramine, 20 μg, injected in the nucleus accumbens also reduced muscimol-induced eating but had no effect on the food intake of starved rats. The results suggest an important role of 5-HT in the nucleus accumbens in the control of certain types of hyperphagia in rats.

It has recently been shown that (+)-fenfluramine, but not (+)-amphetamine, inhibited eating caused by muscimol injected in the nucleus raphe dorsalis (NRD) (Borsini et al 1983). Since, unlike other phenethylamines, (+)-fenfluramine depresses food intake in rats via 5-HT (Samanin & Garattini 1982; Samanin 1983), it was suggested that 5-HT mechanisms might be involved in (+)-fenfluramine's ability to inhibit muscimol-induced eating. In the same study (Borsini et al 1983) penfluridol, which blocks dopamine receptors, was also found to inhibit the feeding response elicited by muscimol, and in a subsequent experiment fluphenazine injected in the nucleus accumbens, but not in the striatum, reduced the effect of muscimol (Bendotti et al, unpublished observations). These findings suggest that muscimol-induced eating is mediated by the DA mesolimbic system. In view of the evidence that 5-HT inhibits DA activity in the nucleus accumbens (Costall et al 1976), one mechanism by which (+)-fenfluramine inhibits muscimol's effect might be to increase 5-HT transmission in this area.

This hypothesis was examined by studying muscimol and starvation-induced eating in rats injected with 5-HT in the nucleus accumbens and corpus striatum. In one experiment, (+)-norfenfluramine, the active metabolite of (+)-fenfluramine (Garattini et al 1979), was injected in the nucleus accumbens and its effect on muscimol- and starvation-induced eating studied.

Materials and methods

Male CD-COBS rats (Charles River, Italy), 230–280 g at the time of surgery, were housed 4 per group at a constant room temperature (21 ± 1 °C) and relative humidity (60%) with free access to food and water. Altromin MT pellets for rats (Rieper, Italy) were used for all experiments.

Implantation of cannulae. Under diethyl ether anaesthesia, rats were stereotaxically implanted with chronic bilateral guide cannulae constructed from 23 gauge stainless-steel tubing in Plexiglass holders, placed with their tip 2 mm above the target area. Stainless-steel stylets, 30 gauge, as long as the guide, kept the guides patent until the animals were given intracerebral injections 7–10 days later.

The rats were accustomed to handling before testing and on the day of the test the stylets were withdrawn and replaced by bilateral injection units (30 gauge stainless-steel tubing) terminating 2 mm below the tip of the guides. The following stereotaxic coordinates from the König & Klippel Rat Brain Atlas (1963) were used: A 9410, L ± 1·5, V − 1 for nucleus accumbens (NA) and A 8620, L ± 2·2, V − 0·6 for caudate putamen (CP).

Muscimol injections in the NRD. Seven or ten days after brain cannula implantation, rats were anaesthetized with diethyl ether and positioned on the stereotaxic instrument for muscimol injection into NRD. The following stereotaxic coordinates were used: A = 0·35, L = 0, V = 0·6 (König & Klippel 1963).

Lignocaine was applied to the suture to prevent local pain that might influence the rats behaviour. Muscimol 100 ng (Biosearch, San Raphael, CA, USA) dissolved in distilled water was injected through an Agla syringe in a volume of 0·5 μl in 1 min. Control animals received an equal volume of the vehicle.

Food intake in muscimol-injected or food-deprived rats. Twenty minutes after muscimol injection the animals were put in cages containing a weighed amount of food. The food consumed in 30 min was measured to the nearest 0·1 g. The mean food intake of all rats injected with vehicle into NRD was 0·5 ± 0·2 g.

For experiments with food-deprived animals, the subjects were trained for 15 days to eat their food during 4 out of 24 h (11.00 am to 3 pm). On day 6–7 of this training they were stereotaxically implanted with bilateral chronic cannulae according to the methods described above.

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On the day of testing, day 15, the rats were put singly in cages containing a weighed amount of food and intake during the first 30 min was measured to the nearest 0·1 g. In both experiments the amount of food eaten was corrected for spillage.

Drugs. 5-Hydroxytryptamine creatinine sulphate (Fluka A.G., Buchs S.G.—Switzerland) was dissolved in ascorbic acid (0·04% w/v) and injected 30 min after intraperitoneal injection of 15 mg kg^{-1} of pargyline HCl (Aldrich Europe, Beerse, Belgium), a monoamine oxidase inhibitor, to reduce metabolic amine degradation. Control animals received the same dose of pargyline 30 min before the intracerebral injection of ascorbic acid. Doses of 5-HT were calculated as free base. (+)-Norfenfluramine HCl (Servier, Neully sur Seine, France) was dissolved in saline. Doses were calculated as salt. All solutions were delivered bilaterally through a 10 µl Hamilton microsyringe coupled to injection units in a volume of 2 µl each side in 2 min, just before access to food. Control animals received an equal volume of the vehicle.

Histological examinations. After completion of the experiments, animals were decapitated, brains were immediately frozen in crushed dry ice and 40 µm sections cut in a cryostat. Under these conditions the traces of the needle and of the cannulae were clearly seen and served to locate the site of injection. Only data from rats in which the needle was exactly placed in NRD and the cannulae were exactly located bilaterally in nucleus accumbens and caudate putamen were included in the results.

Statistics. Data were analysed by Dunnett's two-tailed test for multiple comparison.

Results

As previously reported (Przewlocka et al 1979; Borsini et al 1983), muscimol, injected into the NRD, caused intense eating associated with strong locomotion, rearing, stereotyped sniffing and occasional gnawing and licking of the floor of the cage. As shown in Table 1, a dose-related reduction of muscimol-induced eating was seen after bilateral injections of 5-HT in the nucleus accumbens, but no effect was observed when larger doses of 5-HT were injected in the striatum. No attempt was made to quantify behavioural activation, but observation of the animals during the feeding test indicated that it was not apparently modified.

Eating induced by starvation was not changed by any dose of 5-HT injected in either the nucleus accumbens or the caudate putamen. As shown in Table 2, 20 µg (+)-norfenfluramine significantly reduced muscimol-induced eating, but had no effect on the food intake of starved rats; 10 µg had no effect in either condition.

Table 1. Effect of 5-HT injected bilaterally into the nucleus accumbens or into caudate putamen on eating induced by muscimol injection into the NRD or by starvation.

Treatment	Dose (µg/2 µl)	Eating (g/30 min) induced by Muscimol	Starvation
Nucleus accumbens			
Vehicle	—	4·2 ± 0·6	5·7 ± 0·9
5-HT	2·2	4·6 ± 1·3	5·6 ± 0·5
5-HT	4·4	2·0 ± 0·7*	4·5 ± 0·5
5-HT	8·8	0·5 ± 0·3**	5·0 ± 1·0
Caudate putamen			
Vehicle	—	5·5 ± 1·1	3·5 ± 2·4
5-HT	4·4	5·1 ± 1·0	4·4 ± 0·8
5-HT	8·8	5·7 ± 0·9	4·3 ± 0·6
5-HT	17·6	6·2 ± 0·7	—

Values are means ± s.e. of 5–8 rats.
*$P < 0·05$, **$P < 0·01$, versus vehicle. Dunnett's test (two tailed).

Table 2. Effect of (+)-Norfenfluramine injected bilaterally into the nucleus accumbens on eating induced by muscimol injection into the NRD or by starvation.

Treatment	Dose (µg/2 ml)	Eating (g/30 min) induced by Muscimol	Starvation
Vehicle	—	4·5 ± 0·4	6·4 ± 0·4
(+)-Norfenfluramine	10	3·9 ± 0·9	6·0 ± 0·5
(+)-Norfenfluramine	20	1·6 ± 0·4**	5·4 ± 0·2

Values are mean ± s.e. of 4–6 rats.
**$P < 0·01$ versus vehicle. Dunnett's test (two tailed).

Discussion

5-HT injected into the nucleus accumbens, but not into the striatum, significantly reduced the feeding response of normally fed rats elicited by muscimol injected in the NRD. In contrast, food intake of deprived rats was not affected by 5-HT injection into either area. These findings confirm a previous suggestion (Borsini et al 1983) that eating induced by muscimol or starvation involves different mechanisms. There is evidence that 5-HT in the hypothalamus acts by inhibiting feeding reponses elicited by factors other than muscimol, including starvation (Leibowitz & Papadakos 1978; Lehr & Goldman 1973).

As regards the mechanism by which 5-HT in the nucleus accumbens inhibited muscimol-induced eating, injection of fluphenazine, which blocks dopamine receptors, in this area, also reduced the effect of muscimol. Specific changes in dopamine release and synthesis have been found in the nucleus accumbens of rats which had received muscimol in the NRD (Bendotti et al, unpublished observations). In view of the evidence that 5-HT in the nucleus accumbens inhibits the activity of dopamine (Costall et al 1976), it is likely that 5-HT in the nucleus accumbens inhibited muscimol-induced eating by changing dopamine function in this area. However, 5-HT may block the effect of muscimol by mechanisms other than dopamine.

(+)-Norfenfluramine injection into the nucleus accumbens also reduced the effect of muscimol, but had no effect on food intake of starved rats. This suggests that the effect of systemically injected (+)-fenfluramine (Borsini et al 1983) may be mediated by an increase of 5-HT transmission in the nucleus accumbens. Previous studies with intracerebral injections of fenfluramine or norfenfluramine indicated that the lateral hypothalmus, the neostriatum and the nucleus interstitialis striae terminalis are involved in the depression of feeding caused by fenfluramine in deprived rats (Blundell & Leshem 1973; Broekkamp et al 1975). Assuming that 5-HT mediates the effect of intracerebrally injected fenfluramine, activation of 5-HT mechanisms in different brain areas may be involved in drug-induced inhibition of feeding in different conditions.

In conclusion, the present study provides evidence that the nucleus accumbens may be an important area for the inhibitory effect of 5-HT and '5-hydroxytryptaminergic' drugs on particular types of overeating not associated with nutritional deficits.

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Effects of chloroquine and didesethylchloroquine on rabbit myocardium and mitochondria

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The effects of chloroquine and didesethylchloroquine on the rabbit isolated perfused heart and on calcium binding and accumulating ability of the heart mitochondria were investigated. The drugs produced negative chronotropy, negative inotropy and a decrease in coronary flow rate in the isolated perfused myocardium. Both chloroquine and didesethylchloroquine significantly decreased mitochondrial calcium binding and accumulation. These results suggest that the cardiodepressant actions of chloroquine could be due in part to alterations in the calcium accumulating abilities of the mitochondrial membranes, and that didesethylchloroquine, among other metabolites, does contribute significantly to the total observed effect of chloroquine on the cardiovascular system.

The therapeutic use of chloroquine has resulted in death from poisoning by the drug (Nelson & Conlin 1950; Sanghvi & Mathur 1965). This has been attributed to an effect on the cardiovascular system (Sanghvi & Mathur 1965; Michael & Aiwazzadeh 1970); chloroquine produces negative chronotropic and inotropic effects in animals (Ojewole 1976; Sofola 1980). However, there have been no reports on the molecular basis of action of chloroquine on either the myocardium or any part of the cardiovascular system. Also, there have been no studies on the contribution of some or all of its metabolites to the action. Since subcellular membranes have been considered to play a role in the regulation of heart function and since alterations in their activities are believed to be involved in the development of myocardial contractile failure (Dhalla et al 1977), the present experiments were undertaken to examine the actions of chloroquine on the isolated perfused myocardium and the subcellular organelles invested with double membranes.

In working with the mitochondria, we took advantage of the configurational changes and the attendant changes in light scattering which result from the binding of Ca^{2+} ions to the mitochondrial inner membrane (Kirtland & Baum 1972).

Methods

In one set of experiments, rabbits, 1·0–1·5 kg, were

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