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Eating caused by neuropeptide-Y injection in the paraventricular hypothalamus: response to (+)-fenfluramine and (+)-amphetamine in rats

C. BENDOTTI, S. GARATTINI AND R. SAMANIN*

Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milano, Italy

(+)-Fenfluramine and (+)-amphetamine have been compared for their ability to reduce food intake in food-deprived rats or eating caused by injecting neuropeptide-Y in the paraventricular hypothalamus of free feeding rats. (+)-Fenfluramine at doses ranging from 0·625 to 5 mg kg^{-1} reduced eating caused by neuropeptide-Y more effectively than it did the food intake of food-deprived rats, whereas (+)-amphetamine (dose range 0·625–2·5 mg kg^{-1}) reduced both types of eating to a similar extent. The results confirm that (+)-fenfluramine, although less potent than (+)-amphetamine in reducing eating by food-deprived rats, markedly reduces overeating caused by various endogenous substances or stress in free feeding rats. The physiological significance of the neuropeptide-Y-induced eating and its control by (+)-fenfluramine remains to be elucidated.

There is evidence that the effect of anorectic drugs may depend on the mechanisms primarily involved in a particular form of overeating. Thus, hyperphagia associated with behavioural activation such as that caused by tail pinch or muscimol injection in the nucleus raphe dorsalis has been found to be resistant to treatment with (+)-amphetamine but easily controlled by agents enhancing 5-hydroxytryptamine (5-HT) transmission (Antelman et al 1978; Borsini et al 1983).

Similar findings were reported for overeating caused by systemically administered insulin (Carruba et al 1985). On the basis of the fact that dopamine (DA) receptor antagonists blocked eating caused by tail-pinch, muscimol and insulin (Antelman et al 1975; Zis & Fibiger 1975; Borsini et al 1983) but not that elicited by starvation or 2-deoxy-D-glucose (Muller et al 1972), this latter condition being sensitive to the anorectic effect of (+)-amphetamine (see Carruba et al 1985), it has been suggested that hyperphagias mediated by activation of central DA mechanisms may be less affected by (+)-amphetamine (Borsini et al 1983), a drug that increases central DA function by enhancing its release from nerve terminals and inhibiting its reuptake (Samanin & Garattini 1982).

Extremely powerful and long-lasting stimulation of feeding was recently reported in rats injected with neuropeptide-Y (NPY) in the paraventricular hypothalamus (PVH) (Stanley & Leibowitz 1985). Since the effect of NPY was blocked by the DA antagonist haloperidol (Morley & Levine 1985), this may be another case of overeating preferentially affected by agents using 5-HT mechanisms. The aim of the present study was to verify this by studying the effects on feeding caused by starvation or NPY injection in the PVH of two prototype drugs (+)-fenfluramine and (+)-amphetamine, using, respectively, 5-HT and catecholamines to cause anorexia in rats (Samanin & Garattini 1982).

* Correspondence.

MATERIALS AND METHODS

Male or female Sprague-Dawley rats (Charles River, Italy), 200–300 g, were housed at a constant room temperature (21 ± 1 °C) and relative humidity (60%) with free access to food and water. A standard formula diet (Altromin MT pellets for rats, Rieper, Italy) was used.

Effect of anorectics on food intake of starved rats

Rats were trained over 2 weeks to eat their food during a daily 4 h period (from 10 00 to 14 00 h) with water freely available. On the day of testing they were injected with (+)-fenfluramine hydrochloride or (+)-amphetamine sulphate and 15 min after injection a weighed amount of food was placed in each cage. One hour later animals were removed and food was reweighed to the nearest 0·1 g. The difference, corrected for spillage, constituted the measure of food intake.

Effect of anorectics on NPY-induced hyperphagia in sated rats

Under pentobarbitone (50 mg kg^{-1} i.p.) anaesthesia rats were stereotaxically implanted with chronic monolateral guide cannulae constructed from 23 gauge stainless steel tubing placed with the tip 3 mm above the target area (paraventricular hypothalamus). Stainless steel 30 gauge stylets, as long as the guide, kept the guides patent until the animals were given intracerebral injections of NPY 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 3 mm below the tip of the guides. The following stereotaxic coordinates were used: A 5660; L 0·2; H 2·0 (König & Klippel 1963).

On the day of the experiment the animals were habituated to the test cages (Perspex cages with a grid and blotting paper on the floor) for 1 h before testing, with food freely available on the cage floor. (+)-Fenfluramine and (+)-amphetamine were injected intraperitoneally 15 min before NPY. Immediately after the intracerebral injection of NPY the rats were placed in the test cages, with a weighed amount of food on the floor. Food intake corrected for spillage was measured for 1 h.

On completion of the experiments, animals were killed and the brains were removed and put in 10% formalin solution. After 3–4 days 40 μm sections were cut according to standard histological methods.

Statistical analysis

The data were statistically analysed by analysis of variance and post-hoc comparison with the control group was done using Dunnett's test.

Drugs and sources

Neuropeptide Y (Sigma, St Louis, MO, USA), (+)-fenfluramine hydrochloride (Servier, Neully sur Seine, France) and (+)-amphetamine sulphate (Recordati, Milan, Italy) were dissolved in saline. The doses of (+)-fenfluramine and (+)-amphetamine were expressed as the salt.

RESULTS

Most injection sites where 235 pmoles NPY elicited a reliable eating response were found to be in the PVH. In some animals the tip of the cannula was very close to the third ventricle and presumably part of the infused solution had leaked through it. Data from these animals were not included in the results.

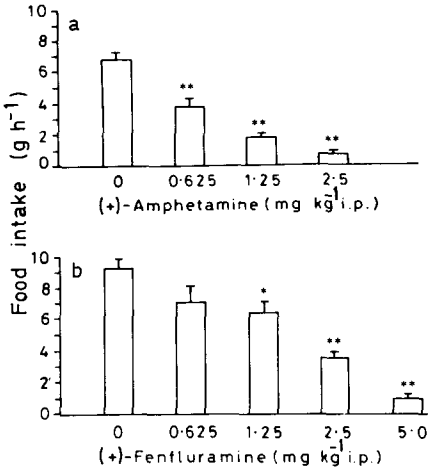

FIG. 1. Dose-response of (a) (+)-amphetamine and (b) (+)-fenfluramine on food-intake of food-deprived rats. The bars represent food intake (g h^{-1}), mean ± s.e. of 6 rats. (+)-Fenfluramine or (+)-amphetamine was injected 15 min before rats had access to food. Data were analysed by analysis of variance and post-hoc comparison with the control groups using Dunnett's test. $*P < 0·05$; $**P < 0·01$ vs saline group.

As shown in Fig. 1, both the drugs dose-dependently reduced food intake of food-deprived rats. The minimal effective dose of (+)-fenfluramine was 1·25 mg kg^{-1} while (+)-amphetamine, 0·625 mg kg^{-1}, significantly reduced eating. Fig. 2 shows the effects on eating caused by NPY injection in the PVH of free-feeding rats. (+)-Fenfluramine, 0·625 mg kg^{-1}, which had no effect on eating by food-deprived rats, caused a more than 50% reduction in eating induced by NPY, while the same dose of (+)-amphetamine, which reduced food intake of food-deprived rats by about 50%, had no effect. There was not much difference between the effects of 0·625 and 1·25 mg kg^{-1} (+)-fenfluramine but at 2·5 mg kg^{-1} it almost completely abolished NPY-induced eating. The eating induced by NPY injection in the PVH was also markedly reduced by 1·25 mg kg^{-1} (+)-amphetamine.

DISCUSSION

The major finding of the present study is that (+)-fenfluramine, a drug using 5-HT to inhibit feeding (Garattini et al 1979; Borsini et al 1982), markedly reduced hyperphagia caused by injection of NPY in the PVH of free-feeding animals. It has been reported that 5-HT injected in the PVH causes dose-related reduction of food intake and the effect is much greater on overeating caused by injection of noradrenaline into the PVH (Leibowitz 1980). On

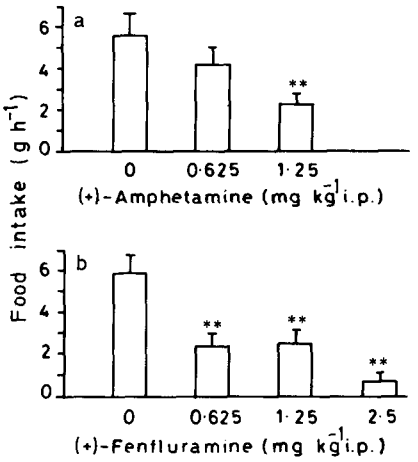

FIG. 2. Effect of (a) (+)-amphetamine and (b) (+)-fenfluramine on hyperphagia induced by microinjection of neuropeptide Y (NPY) in the paraventricular hypothalamus of free feeding rats. The bars represent food intake (g h^{-1}), mean ± s.e. of 7–9 rats. NPY (235 pmol/0·5 μL) dissolved in saline was injected just before testing. Mean food intakes of control rats receiving intracerebral saline were 0·5 ± 0·1 and 0·8 ± 0·4 respectively for experiments on (+)-fenfluramine and (+)-amphetamine. Data were analysed by analysis of variance and post-hoc comparison with the control group using Dunnett's test. $^{**}P < 0·01$ vs saline group.

the basis of some similarities in the effects on meal patterns of 5-HT injected into the PVH and peripheral administration of fenfluramine, it has been suggested that the PVH may be a common site of action for centrally and peripherally administered 5-HT agonists (Leibowitz & Shor-Posner 1986). NPY-induced hyperphagia is not mediated by an action on PVH α-adrenoceptors involved in feeding stimulation (Stanley & Leibowitz 1985), but nevertheless it is markedly inhibited by agents enhancing 5-HT transmission in the brain. This suggests that 5-HT in the PVH exerts an inhibitory action on feeding stimulation caused by endogenous substances such as noradrenaline and NPY.

5-HT and (+)-norfenfluramine were recently found to inhibit dopamine-dependent feeding when injected in the nucleus accumbens (Bendotti et al 1986a, b), and hyperphagia caused by NPY injected in the PVH was reported to be blocked by haloperidol, a potent dopamine receptor blocker (Morley & Levine 1985). The possibility therefore cannot be excluded that sites in some extrahypothalamic areas contribute to the inhibitory effect of (+)-fenfluramine on feeding caused by NPY in the PVH.

(+)-Fenfluramine showed less activity on eating by food-deprived rats. Since the lateral hypothalamus, an area of crucial importance in feeding control (Hoebel 1984), has been shown to be relatively insensitive to direct 5-HT stimulation (Leibowitz 1980), the moderate activity of (+)-fenfluramine in food-deprived rats may reflect its relative ineffectiveness in controlling feeding at this level.

Unlike overeating caused by tail pinch and muscimol injection in the dorsal raphe (Antelman et al 1975; Borsini et al 1983), which are particularly sensitive to drugs enhancing 5-HT transmission and completely resistant to the effect of (+)-amphetamine, eating caused by PNY injection in the PVH was significantly reduced by (+)-amphetamine, although to a lesser extent than eating by food-deprived rats. Since the anorectic effect of (+)-amphetamine is commonly attributed to its ability to enhance catecholamine transmission in the lateral hypothalamus (Leibowitz 1980), this mechanism may contribute to its effect on NPY-induced eating.

A recent study showed that a feeding response is elicited by injecting NPY in various hypothalamic areas, including the lateral hypothalamus (Stanley et al 1985), thus the sensitivity of NPY-induced eating to (+)-amphetamine may differ in the various hypothalamic areas where NPY stimulates feeding.

The present study confirms previous findings (Borsini et al 1983) that (+)-fenfluramine is less potent than (+)-amphetamine in reducing eating by food-deprived rats, probably because of its relative inability to control mechanisms in the lateral hypothalamus that favour feeding behaviour. Overeating caused by NPY in the PVH, like that of noradrenaline in the same area or that mediated by an increase of dopaminergic transmission in the corpus striatum (tail pinch) (Antelman et al 1978) and nucleus accumbens (muscimol injection in the dorsal raphe; Borsini et al 1983; Bendotti et al 1986a, b) is particularly sensitive to the action of (+)-fenfluramine. The importance of these findings for the pharmacological treatment of feeding disorders in humans is not known.

REFERENCES

Antelman, S. M., Szechtman, H., Chin, P., Fisher, A. E. (1975) Brain Res. 99: 319–337

Antelman, S. M., Caggiula, A. R., Block, C. A., Edwards, D. J. (1978) Ibid. 143: 580–585

Bendotti, C., Berettera, C., Invernizzi, R., Samanin, R. (1986a) Pharmacol. Biochem. Behav. 24: 1089–1093

Bendotti, C., Garattini, S., Samanin, R. (1986b) J. Pharm. Pharmacol. 38: 541–543

Borsini, F., Bendotti, C., Aleotti, A., Samanin, R., Garattini, S. (1982) Pharmacol. Res. Commun. 14: 671–678

Borsini, F., Bendotti, C., Przewlocka, B., Samanin, R. (1983) Eur. J. Pharmacology 94: 109–115

Carruba, M. O., Ricciardi, S., Spano, P. F., Mantegazza, P. (1985) Life Sci. 36: 1739–1749

Garattini, S., Caccia, S., Mennini, T., Samanin, R., Consolo, S., Ladinsky, H. (1979) Curr. Med. Res. Opin. 6: suppl. 1, 15–27

Hoebel, B. G. (1984) in: Stunkard, A. J., Stellar, E. (eds) Eating and its Disorders. Raven Press, New York, pp 15–38

König, J. F. R., Klippel, R. A. (1963) The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. William & Wilkins, Baltimore

Leibowitz, S. F. (1980) in: Morgane, P. J., Panksepp, J. (eds) Handbook of the hypothalamus Vol. 3 part A. Behavioral studies of the hypothalamus, Marcel Dekker, New York, pp 299–437

Leibowitz, S. F., Shor-Posner, G. (1986) in: Carruba, M. O., Blundell, J. E. (eds) Pharmacology of eating disorders: Theoretical and clinical developments. Raven Press, New York, pp 29–49

Morley, J. E., Levine, A. S. (1985) Ann. Rev. Pharmacol. Toxicol. 25: 127–146

Muller, E. E., Cocchi, D., Mantegazza, P. (1972) Am. J. Physiol. 223: 945–950

Samanin, R., Garattini, S. (1982) in: Silverstone, T. (ed.) Drugs and appetite, Academic Press, London, pp 23–39

Stanley, B. G., Leibowitz, S. F. (1985) Proc. Natl. Acad. Sci. USA 82: 3940–3943

Stanley, B. G., Chin, A. S., Leibowitz, S. F. (1985) Brain Res. Bull. 14: 521–524

Zis, A. P., Fibiger, H. C. (1975) Psychopharmacologia (Berlin) 43: 63–68