The effects of intracerebral norfenfluramine on body temperature of the rat

Z. L. KRUK, *Department of Pharmacology and Therapeutics, The London Hospital Medical College, Turner Street, London, E1 2AD, U.K.*

While it is well established that the anorectic agent fenfluramine can be de-ethylated *in vivo* to norfenfluramine (desethylfenfluramine) (Bruce & Maynard, 1968; Morgan, Cattabeni & Costa, 1972) it still remains to be established if one or both compounds mediate the anorectic and behavioural responses observed.

Broekkamp, Weemas & van Rossum (1975) have demonstrated anorexia following intracerebral injections of the norfenfluramines into neostriatum or nucleus interstitialis of the stria terminalis while fenfluramine injected at these sites was inactive, thus suggesting that the anorectic effect of fenfluramine is mediated by norfenfluramine. Kruk (1973) showed that intracerebroventricular (i.c.v.) injections of norfenfluramine reduced food intake in hungry rats, while similar injections of fenfluramine were inactive (unpublished observation). A reduction in core temperature following i.c.v. injection of norfenfluramine in sheep has been reported by Bligh, Sharman & others (1974) but these authors do not describe the effects of fenfluramine. The effects of i.c.v. injections of fenfluramine and norfenfluramine on the body temperature of the rat are now reported.

A permanent cannula guide was implanted in the left lateral ventricle of male Sprague–Dawley rats. The rats were housed individually in a room maintained at 20–22° and a 12 h light, 12 h dark cycle. A minimum of one week was allowed for recovery after surgery and the animals were subsequently used for injection once weekly. Drugs were dissolved in saline and injected in a volume of 10 μl, controls received saline only. Body core (oesophageal) and skin (tail) temperatures were measured using a multichannel electric thermometer, 60 and 30 min before the i.c.v. injection, immediately before injection (time 0) and then 2, 5, 10, 15, 25, 40, 60 and 90 min after. Temperature changes refer to differences from time 0. The results are expressed as mean responses from at least six animals, degrees of significance were assessed using a non-paired Student's *t*-test.

Saline (10 μl) i.c.v. had little effect on either body core of skin temperature. The handling procedure without saline injection produced similar effects. Fenfluramine i.c.v. resulted in a small rise in core temperature and a variable response in the skin temperature. Neither of these effects was significantly different from the response following saline injection alone (Fig. 1a).

Norfenfluramine (10, 50, 100 μg per rat, i.c.v.) resulted in a dose-dependent fall in core temperature. Ten min after i.c.v. injections of norfenfluramine (10 μg per rat) the core temperature was reduced compared with saline controls (t = 4·8 d.f. 10 $P<0{\cdot}001$) while the corresponding skin temperature was increased compared with saline control (t = 3·36, d.f. 10 $P<0{\cdot}01$). With higher doses of norfenfluramine greater falls in core temperature and greater rises in skin temperature were observed. The core and skin temperatures returned to within control values within 90 min after norfenfluramine injection (Fig. 1b).

The present results show that when injected by the intracerebroventricular route, fenfluramine is inactive in causing a fall in body core temperature accompanied by a rise in skin temperature. The metabolite of fenfluramine-norfenfluramine is active in this respect,

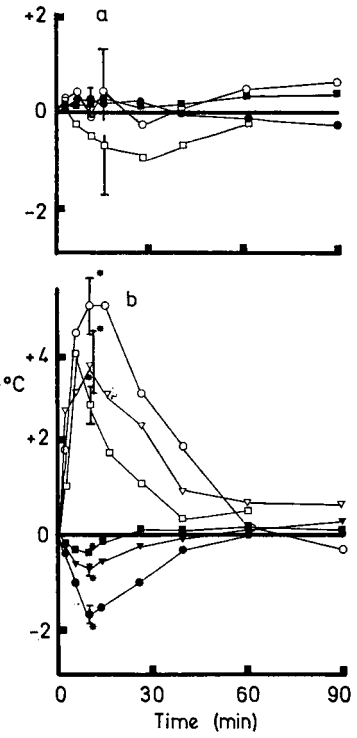

FIG. 1. Mean effect on body core (closed symbols) and skin temperature (open symbols) following i.c.v. injection of a, 0·9% w/v saline (●)-10 μl per rat or fenfluramine (■) -100 μg per rat, in the conscious rat, and b, norfenfluramine (■)-10 μg per rat, (▼)-50 μg per rat, and (●)-100 μg per rat, in the conscious rat. * $P<0{\cdot}01$ when compared with saline controls. y axis—Change in temperature from time 0 (°C).

the fall in core temperature and rise in skin temperature being dose-dependent.

When the results of other workers comparing fenfluramine and norfenfluramine are examined, a similar pattern emerges. Thus in relation to body temperature changes in the sheep, i.c.v. injected norfenfluramine is active in causing a fall in body core temperature (Bligh & others, 1974), while fenfluramine injected i.c.v. is inactive in causing body core temperature changes (Bligh; personal communication).

When the effects of fenfluramine and norfenfluramine administered intracerebrally are examined for their anorectic activity, results analagous to those for body temperature changes are seen. Kruk (1973) showed that norfenfluramine caused a decrease in feeding following i.c.v. injection, while fenfluramine did not (Kruk, unpublished observation). Broekkamp & others (1975) injected both fenfluramine and norfenfluramine into the neostriatum and the nucleus interstitialis of the stria terminalis of the rat, and found that while norfenfluramine caused a dose-dependent anorectic response, fenfluramine was inactive.

In man, the mydriasis caused by topical application of norfenfluramine to the eye was not observed when fenfluramine was similarly applied (Kramer, Rubicek & Turner, 1973). Furthermore, Kramer & others (1973) found that following systemic administration of fenfluramine, the mydriasis observed was more closely related to the plasma concentrations of norfenfluramine than fenfluramine.

All the results quoted above suggest that the actions of fenfluramine are due to its active metabolite norfenfluramine. Other workers, however, suggest that fenfluramine itself may be centrally active, but the evidence on which they base their conclusions is not completely convincing. Thus, from experiments where anorexia was observed in response to fenfluramine in rats pretreated with the liver microsomal enzyme inhibitor SKF 525A, Garattini, Buczko & others (1975) concluded that fenfluramine can mediate the central anorectic responses in rats. These authors, however, neither measured plasma concentrations of fenfluramine and norfenfluramine, nor did they assess the degree of liver microsomal enzyme inhibition so the experiments cannot be taken as good evidence for fenfluramine being able to cause the anorectic response in the rat.

Where plasma concentrations of fenfluramine and norfenfluramine have been measured, the anorectic response would appear to be best related with the combined plasma concentrations of fenfluramine and norfenfluramine (Blundell, Campbell & others, 1975). However a firm conclusion could not be drawn from the results as the plasma concentrations of norfenfluramine following injections of fenfluramine were not described. In a further series of experiments, Blundell & Campbell (1975) measured plasma concentrations of norfenfluramine after intraperitoneal administration of norfenfluramine but the relation between plasma concentration of norfenfluramine and anorectic response is not shown so again it is not possible to say on the basis of the plasma measurements whether the total anorectic response can be accounted for in terms of norfenfluramine activity.

In vitro studies show that both fenfluramine and norfenfluramine are capable of altering biochemical mechanisms. Thus both substances increase the uptake of glucose into skeletal muscle (Kirby & Turner, 1974) both can inhibit the uptake and cause the release of 5-hydroxytryptamine from blood platelets (Buczko, de Gaetano & Garattini, 1975) and both can block the uptake and cause the release of dopamine, noradrenaline and 5-HT in rat brain slices (Fuxe, Farnebo & others, 1975) and synaptosomes prepared from rat brain (Kruk & Zarrindast, in preparation). The fact that both fenfluramine and norfenfluramine are active *in vitro* is not surprising, for problems of access in such situations do not arise.

While the present results do not exclude the possibility that at peripheral sites both compounds can exert an effect, the consistency with which it has been demonstrated that when applied directly into the cns, norfenfluramine is active and fenfluramine is not active, argues strongly in favour of the suggestion that the cns responses observed *in vivo* following administration of fenfluramine are due to the active metabolite norfenfluramine.

I thank Servier Laboratories for gifts of fenfluramine and norfenfluramine. July 8, 1976

REFERENCES

BLIGH, J., SHARMAN, D. F., SÜMEGI, I. & SZÉKELY, M. A. (1974). *J. Physiol. Lond.*, **239**, 111P.
BLUNDELL, J. E. & CAMPBELL, D. B. L. (1975). *Br. J. Pharmac.*, **55**, 261P.
BLUNDELL, J. E., CAMPBELL, D. B., LESHAM, M. & TOZER, I. (1975). *J. Pharm. Pharmac.*, **27**, 187–192.
BROEKKAMP, C. L. E., WEEMAES, A. J. M. & VAN ROSSUM, J. M. (1975). *Ibid.*, **27**, 129–130.
BRUCE, R. B. & MAYNARD, W. R. (1968). *J. pharm. Sci.*, **57**, 1173–1175.
BUCZKO, W., DE GAETANO, G. & GARATTINI, S. (1975). *Br. J. Pharmac.*, **53**, 563–569.
FUXE, K., FARNEBO, L. O., HAMBERGER, B. & ÖGREN, S. O. (1975). *Postgrad. med. J.*, **51**, *Suppl.* 1, 35–45.
GARATTINI, S., BUCZKO, W., JORI, A. & SAMANIN, R. (1975). *Ibid.*, **51**, *Suppl.* 1, 27–35.
KIRBY, M. J. & TURNER, P. (1974). *Br. J. Pharmac.*, **50**, 477P.
KRAMER, R., RUBICEK, M. & TURNER, P. (1973). *J. Pharm. Pharmac.*, **25**, 575–576.
KRUK, Z. L. (1973). *Nature, New Biology*, **246**, 52–53.
MORGAN, C. D., CATTABENI, F. & COSTA, E. (1972). *J. Pharmac. exp. Ther.*, **180**, 127–134.