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Effects of Xylamidine on Peripheral 5-Hydroxytryptamine-Induced Anorexia

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EDWARDS, S. AND R. G. STEVENS. *Effects of xylamidine on peripheral 5-hydroxytryptamine-induced anorexia.* PHARMACOL BIOCHEM BEHAV **34**(4) 717–720, 1989.—Rats injected peripherally with 5-hydroxytryptamine (5-HT) showed a dose-dependent decrease in food intake following overnight fasting. The peripheral 5-HT-2 antagonist xylamidine had no effect on food intake when administered alone, but antagonised 5-HT-induced anorexia. However, at the highest dose of 5-HT (5 mg/kg), both doses of xylamidine (1.0 and 2.0 mg/kg) displayed the same degree of antagonism to the anorectic effect, but failed to block it completely. The results are discussed in terms of 5-HT receptor subtypes, and it is suggested that non-5-HT-2 receptors may be partially responsible for the mediation of peripheral 5-HT-induced anorexia.

5-Hydroxytryptamine Xylamidine Food intake 5-HT receptors Anorexia

ALTHOUGH Bray and York (2) suggested a role for peripheral serotonergic systems in the regulation of food intake and eating behaviour, it is only recently that this possibility has been seriously investigated. It is now well established that systemically administered 5-hydroxytryptamine (5-HT), which does not cross the blood-brain barrier (16), produces a dose-dependent reduction in food intake (7,17) which is not due to nonspecific effects such as impairment of locomotor or sensorimotor function or the production of a conditioned taste aversion (17). Furthermore, this anorectic effect is large enough to block both insulin- and 2-deoxyglucose-induced hyperphagias (3).

Further examineration of this anorectic effect has been carried out using the serotonergic drugs clorgyline, methysergide and metergoline (7,17). However, there is considerable evidence that these drugs cross the blood-brain barrier (6, 12, 13, 20), and central serotonergic systems are also known to influence the regulation of food intake [see (1,14) for reviews]. These drugs, therefore, are unsuitable for the study of peripheral serotonergic systems alone, since any outcome of their use may be the result of an interaction between peripheral and central serotonergic systems.

Copp *et al.* (5) produced xylamidine, a 5-HT antagonist which does not cross the blood-brain barrier, and which acts specifically on 5-HT-2 receptors (11). It has been reported that xylamidine attenuates the anorectic effect of systemically administered 5-HT (4,9). Thus, there is now evidence that peripheral 5-HT-2 receptors are important in the mediation of this effect. Fletcher and Burton (9) reported that xylamidine alone had no effect on food intake. However, this was in contradiction to the findings of Clineschmidt *et al.* (4) who found that xylamidine reduced food intake in its own right. Furthermore, Clineschmidt *et al.* reported that the anorectic effect of xylamidine is additive to that produced by the 5-HT agonist 6-chloro-2-[1-piperazinyl]-pyrazine (MK-212) which is thought to act centrally. It is unclear why such disparate findings should emerge.

This series of experiments, therefore, was initiated to investigate the effects of peripheral 5-HT and xylamidine on food intake. In the first experiment, the effects of 5-HT alone and xylamidine alone were examined.

EXPERIMENT 1

Subjects

Twenty-eight male Wistar rats were used (220–320 g). They were housed in individual cages with water available ad lib. Ambient temperature was kept at 22 degrees centigrade, and the animals were maintained on a 12/12 light/dark cycle (lights on 0730–1930).

Drugs

The drugs used were 5-hydroxytryptamine creatinine sulphate (Sigma), and xylamidine tosylate (Wellcome). The dosages used were 1.25, 2.5, and 5.0 mg/kg 5-HT, and 0.5, 1.0 and 2.0 mg/kg xylamidine tosylate (XYL). All drugs were delivered subcutaneously in a 0.9% saline vehicle. Injection volumes were 1.33 ml/kg (XYL) and 0.86 ml/kg (5-HT).

Procedure

Before the experiment, the rats were habituated to a restricted feeding regimen for two weeks. They were presented with standard rat chow at 1430, and this was removed at 1830. Two "mock" treatments were given. These were the same as the experimental procedures except that the two injections were 0.9% saline in all cases.

Each experimental treatment was administered as two injections at 0930 and 1330. The first was either xylamidine or vehicle, and the second was either 5-HT or vehicle. The seven experimental treatments used were 0.5 mg/kg XYL + veh, 1.0 mg/kg

TABLE 1

SUMMARY TABLE OF MEAN FOOD INTAKE (g) PER HOUR FOR EACH TREATMENT OVER TIME

Drug	Dosage (mg/kg)	Hours Post-Food Presentation 1	2	3	4
SAL	0.9%	7.41	2.70	3.82	4.56
5-HT	1.25	7.30	2.82	3.54	4.57
	2.5	5.80	3.23	4.00	4.39
	5.0	4.23	3.14	3.23	4.55
XYL	0.5	7.36	2.88	3.68	4.48
	1.0	7.29	2.89	3.80	4.36
	2.0	7.07	2.88	3.77	4.54

XYL + veh, 2.0 mg/kg XYL + veh, veh + 1.25 mg/kg 5-HT, veh + 2.5 mg/kg 5-HT, veh + 5.0 mg/kg 5-HT, and veh + veh. All rats received all treatments. The order of treatments was determined by a 7 × 7 Latin square, and at least two drug-free days were allowed between experimental treatments.

After the second injection the rats were weighed. Preweighed food-hoppers (containing at least 150 g of food) were presented at 1430, and these were reweighed at 1530, 1630, 1730 and 1830, when the feeding period ended.

Results

Means of food intake over time for each treatment are shown in Table 1. A 7 × 4 two-way within-subjects ANOVA (with treatment and time as factors) yielded significant main and interaction effects. The following values were calculated: for treatment, $F(6,162) = 14.72$, $p<0.0001$; for time, $F(3,162) = 231.18$, $p<0.0001$; for interaction between treatment and time, $F(18,486) = 9.43$, $p<0.0001$. Since the effects of treatment were only significant during the first hour, $F(6,162) = 49.10$, $p<0.001$, post hoc analyses using Scheffé tests were carried out on the 1 hour data (Table 2). Food intake was significantly reduced in the 2.5 and 5.0 mg/kg 5-HT conditions (in each case, $p<0.001$), but not in the 1.25 mg/kg 5-HT condition, as compared to control. Food intake in the 5.0 mg/kg 5-HT condition was significantly lower than in the 2.5 mg/kg 5-HT condition ($p<0.001$). There was no significant difference between food intake in any of the xylamidine conditions as compared to control.

Discussion

The dose-dependent nature of the anorectic effect of systemically administered 5-HT in this experiment supports the observations of previous reports. The failure of xylamidine alone to affect food intake supports the previous observations of Fletcher and Burton (9), rather than Clineschmidt *et al.* (4), and extends the evidence on xylamidine to additional doses.

A second experiment was designed to confirm the antagonism by xylamidine of peripheral 5-HT-induced anorexia and to investigate its characteristics. Two doses of xylamidine were included in an attempt to detect a dose-response relationship for this antagonist effect.

TABLE 2

SUMMARY TABLE OF SCHEFFÉ TEST RESULTS FOR EACH TREATMENT AT 1 HOUR POST-FOOD PRESENTATION

		1	2	3	4	5	6
1	Control	—					
2	1.25 mg/kg 5-HT	ns	—				
3	2.5 mg/kg 5-HT	*	*	—			
4	5.0 mg/kg 5-HT	*	*	*	—		
5	0.5 mg/kg XYL	ns	ns	*	*	—	
6	1.0 mg/kg XYL	ns	ns	*	*	ns	—
7	2.0 mg/kg XYL	ns	ns	*	*	ns	ns

ns: no significant difference.
*$p<0.001$.

TABLE 3

SUMMARY TABLE OF MEAN FOOD INTAKE (g) PER HOUR FOR EACH TREATMENT OVER TIME

1st Inj Drug	Dosage (mg/kg)	2nd Inj Drug	Dosage (mg/kg)	Hours Post-Food Presentation 1	2	3	4
SAL	0.9%	SAL	0.9%	6.98	2.54	2.89	3.36
SAL	0.9%	5-HT	2.5	5.09	2.98	3.38	3.34
SAL	0.9%	5-HT	5.0	3.66	2.52	2.82	4.12
XYL	1.0	5-HT	2.5	6.25	2.39	2.68	4.29
XYL	1.0	5-HT	5.0	5.71	2.09	3.30	3.82
XYL	2.0	5-HT	2.5	6.50	2.11	3.34	3.57
XYL	2.0	5-HT	5.0	5.38	2.25	3.50	3.66

EXPERIMENT 2

Subjects

Twenty-eight male Wistar rats were used (200–250 g). Housing conditions were as described in Experiment 1.

Drugs

The drugs were as previously described; the dosages were 2.5 and 5.0 mg/kg 5-HT and 1.0 and 2.0 mg/kg XYL.

Procedure

The same procedure was used as in Experiment 1.

The first injection was either xylamidine (1.0 or 2.0 mg/kg) or vehicle, and the second was either 5-HT (2.5 or 5.0 mg/kg) or vehicle. The seven experimental treatments used were veh + 2.5 mg/kg 5-HT, veh + 5.0 mg/kg 5-HT, 1.0 mg/kg XYL + 2.5 mg/kg 5-HT, 1.0 mg/kg XYL + 5.0 mg/kg 5-HT, 2.0 mg/kg XYL + 2.5 mg/kg 5-HT, 2.0 mg/kg XYL + 5.0 mg/kg 5-HT, and veh + veh. All rats received all treatments, but in a balanced order.

Results

Means of food intake over time for each treatment are shown in Table 3. A 7 × 4 two-way within-subjects ANOVA (with treatment and time as factors) yielded significant main and interaction effects. The following values were calculated: for treatment, $F(6,162) = 7.62$, $p<0.0001$; for time, $F(3,81) = 185.03$, $p<0.000$; for interaction between treatment and time, $F(18,486) = 7.65$, $p<0.0001$. Once again, the effects of treatment were only significant during the first hour, $F(6,162) = 24.51$, $p<0.001$, so post hoc analyses using Scheffé tests was carried out on the 1 hour data (Table 4). At one hour, food intake was significantly reduced in

TABLE 4

SUMMARY TABLE OF SCHEFFÉ TEST RESULTS FOR EACH TREATMENT AT 1 HOUR POST-FOOD PRESENTATION

		1	2	3	4	5	6
1	Control	—					
2	2.5 mg/kg 5-HT	‡	—				
3	2.5 mg/kg 5-HT + 1 mg/kg XYL	ns	*	—			
4	2.5 mg/kg 5-HT + 2 mg/kg XYL	ns	†	ns	—		
5	5.0 mg/kg 5-HT	‡	†	‡	‡	—	
6	5.0 mg/kg 5-HT + 1 mg/kg XYL	*	ns	ns	ns	‡	—
7	5.0 mg/kg 5-HT + 2 mg/kg XYL	‡	ns	ns	ns	‡	ns

ns: no significant difference.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

both the 2.5 mg/kg and 5.0 mg/kg 5-HT conditions ($p<0.001$ in each case) as compared to controls, and food intake in the 5.0 mg/kg 5-HT condition was significantly lower than in the 2.5 mg/kg 5-HT condition ($p<0.01$).

Xylamidine clearly attenuated the anorectic effect of the 5-HT. At both 2.5 and 5.0 mg/kg 5-HT doses, food intake was significantly higher following pretreatment with 1.0 or 2.0 mg/kg xylamidine as compared with 5-HT alone (at least $p<0.05$ in all cases). However, at both 2.5 mg/kg and 5.0 mg/kg 5-HT doses, there was no significant difference in food intake between the 1.0 and 2.0 mg/kg xylamidine conditions. Furthermore, food intakes in the 5.0 mg/kg 5-HT + 1.0 mg/kg XYL and 5.0 mg/kg 5-HT + 2.0 mg/kg XYL conditions were still significantly lower than the control values (Table 4).

Discussion

It is clear from the results that xylamidine does antagonise peripheral 5-HT-induced anorexia, thus supporting the finding of Fletcher and Burton (9). However, no dose-response relationship has been demonstrated here, since no significant difference in food intake was found between 1.0 and 2.0 mg/kg XYL pretreatment at either dose of 5-HT. Furthermore, food intake for both levels of xylamidine pretreatment plus the higher 5-HT treatment were still significantly lower than controls. It seems unlikely that this can be interpreted in terms of any anorexic effect that xylamidine itself may produce, since no such effect was found in the first experiment above. Neither does it seem likely that this could be interpreted in terms of the pharmacokinetics of xylamidine. It has been shown that the ID_{50} of orally administered xylamidine that antagonises 5-HT-induced foot oedema in the rat is considerably lower when the compound is administered 5 hours, rather than 1 hour, before 5-HT (5,15), that oral or subcutaneous administration of xylamidine 3 hours before 5-HT fully prevented the embryocidal effect of the latter in rats (10), and that intraperitoneal administration of xylamidine 4 hours before 5-HT or 5-HT agonists antagonised peripherally mediated effects on operant conditioning behaviour (18) and hypothermia (19). Therefore, the potency of xylamidine appears greater when it is given several hours before 5-HT. However, there are other possible explanations. The two doses of XYL may be too close together on the rising part of the dose-response curve, or both doses may be on the asymptote of the curve. Further work is underway in this laboratory using a wider range of xylamidine doses to investigate these possibilities. Alternatively, since xylamidine is a specific 5-HT-2 antagonist, this finding may be interpreted in terms of the effects of 5-HT on its receptor subtypes. If the peripheral 5-HT-induced anorectic effect is mediated exclusively by 5-HT-2 receptors, then a ceiling effect for the actions of xylamidine should occur when the anorectic effect has been blocked completely. However, if 5-HT-2 and non-5-HT-2 receptor types are involved, then a ceiling effect for xylamidine could occur when the anorectic effect of 5-HT had been attenuated but not completely blocked. The results of this experiment indicate this may be so.

GENERAL DISCUSSION

It has been shown here that there may be both 5-HT-2 and non-5-HT-2 receptor types present in the periphery responsible for mediating the anorectic effect of systemically administered 5-HT. This raises several questions. Which non-5-HT-2 receptor type(s) might be involved is unknown, but could be characterised using 5-HT antagonists that also do not cross the blood-brain barrier and which are specific to other 5-HT receptor subtypes. Furthermore, given that different 5-HT receptor subtypes may be involved in mediating the anorectic response, could they mediate different components of this response?

A major unanswered question is why xylamidine administered alone does not provoke an increase in food intake. It is established that there are 5-HT receptors in the periphery which mediate a considerable anorectic effect. The location of these receptors is unknown, but the enteric nervous system of the gut and the vagus nerve are possible sites according to Fletcher and Burton (8). Nevertheless, given that such a system exists, then administering a drug that antagonises this system should increase food intake. Furthermore, if this peripheral 5-HT system helps mediate satiety, then antagonism of this system should disrupt satiety. However, as has been described, this did not happen with xylamidine for reasons that are unknown. Xylamidine may have nonspecific behavioural effects that mask its effect on satiety. While nonspecific effects, such as disruption of locomotor activity or drinking behaviour or the production of a conditioned taste aversion, have been ruled out for the anorectic effect of peripheral 5-HT, such effects cannot be ruled out in the case of xylamidine.

To conclude, there is a peripheral serotonergic system which can produce a considerable anorectic effect. This system is antagonised by xylamidine, indicating 5-HT-2 receptors are at least partially responsible for mediating this effect. The results of this experiment suggest that non-5-HT-2 receptors may also be involved, although the location of this system and how it works remain unknown.

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