Effects of the peripheral 5-HT₂ antagonist xylamidine on consummatory behaviors

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It has been reported that xylamidine, a specific peripheral 5-HT₂ antagonist, attenuates, but does not completely block, 5-HT-induced anorexia, and that this effect may be mediated by both 5-HT₂ and non-5-HT₂ receptor subtypes. To determine whether this incomplete antagonism was a consequence of xylamidine's being given at suboptimal doses, the dose-response characteristics of xylamidine on the anorexia induced by a dose of 5 mg/kg of 5-HT was investigated; the asymptote of the dose-response curve began at about 0.75 mg/kg. A second reason for the incomplete attenuation of 5-HT-induced anorexia would be that xylamidine has confounding nonspecific behavioral and/or ingestive effects. Xylamidine was found not to alter gross drinking behavior, and in a 2-bottle test, it did not, unlike lithium chloride, produce a conditioned taste aversion. The effects of xylamidine on drinking behavior and conditioning cannot account for the previously reported failure of xylamidine to completely block peripheral 5-HT-induced anorexia, and the possibility of non-5-HT₂ receptors' being partially responsible for the mediation of this anorectic effect remains open.

Systemically administered 5-hydroxytryptamine (5-HT), which does not cross the blood-brain barrier (Olfendorf, 1971) and must therefore be acting peripherally, produces a dose-dependent reduction in food intake (Bray & York, 1972; Clineschmidt, McGuffin, Pflueger, & Totaro, 1978; Edwards & Stevens, 1989; Fletcher & Burton, 1984; Pollock & Rowland, 1981). This effect has been investigated by using xylamidine, a specific peripheral 5-HT₂ antagonist (Copp, Green, Hodson, & Randall, 1967; Fuller, Kurz, Mason, & Cohen, 1986). Since xylamidine administered 4 h before 5-HT has been found to attenuate, but not completely block, peripheral 5-HT-induced anorexia, it has been suggested that peripheral 5-HT-induced anorexia is mediated by both 5-HT₂ and non-5-HT₂ receptor subtypes (Edwards & Stevens, 1989). The long interval between xylamidine and 5-HT administration was allowed because the potency of xylamidine appears greater when given several hours before 5-HT. It has been shown that the ID₅₀ of orally administered xylamidine that antagonizes 5-HT-induced foot edema in the rat is considerably lower when the compound is administered 5 h, rather than 1 h, before 5-HT (Copp et al., 1967; Mawson & Whittington, 1970); that oral or subcutaneous administration of xylamidine 3 h before 5-

HT fully prevented the embryocidal effect of the latter in rats (Fraser, 1970); and that intraperitoneal administration of xylamidine 4 h before 5-HT or 5-HT agonists antagonized peripherally mediated effects on operant conditioning behavior (Winter, 1969) and hypothermia (Carter & Leander, 1980; Winter, 1972). However, before the suggestion of non-5-HT₂ receptor involvement can be accepted, two alternative explanations that first must be eliminated are the possibilities that insufficient xylamidine dosages have previously been used or that the incomplete antagonism of xylamidine may arise from the drug's having nonspecific behavioral effects that may interact with its antagonistic effect on peripheral 5-HT-induced anorexia. These questions are addressed in the following experiments.

EXPERIMENT 1

Dose-Response Relationship for the Effects of Xylamidine on 5 mg/kg 5-HT-Induced Anorexia

The first possible alternative explanation for xylamidine's apparent failure to completely block peripheral 5-HT-induced anorexia is that the dosages of xylamidine used in the previous studies may have been insufficient. This can be tested by determining the dose-response characteristic of xylamidine on 5-HT-induced anorexia. Edwards and Stevens (1989) reported that there is no significant difference between the effects of 1 and 2 mg/kg xylamidine on 5 mg/kg 5-HT anorexia in rats, so here xylamidine doses up to 1 mg/kg are tested on 5 mg/kg 5-HT anorexia to discover whether the asymptote of the dose-response curve begins prior to the 1 mg/kg dosage.

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Method

Subjects. Twenty male Wistar rats were used (205–300 g). They were housed in individual cages with water available ad lib. Ambient temperature was kept constant at 22°C, and the animals were maintained on a 12:12-h light:dark cycle (lights on 0730–1930 h).

Drugs. The drugs used were 5-hydroxytryptamine creatinine sulphate (Sigma) and xylamidine tosylate (Wellcome). The dose of 5-HT was 5.0 mg/kg, and xylamidine was administered at doses of 0, 0.25, 0.5, 0.75, and 1.0 mg/kg. All drugs were delivered subcutaneously in a 0.9% saline vehicle at injection volumes of 1.33 ml/kg for xylamidine and 0.86 ml/kg for 5-HT.

Procedure. Before the experiment, the rats were habituated to a restricted feeding regimen for 2 weeks. They were presented with standard rat chow at 1430 h, and this was removed at 1830 h. Two "mock" treatments were given. These were the same as the experimental treatments, except that the two injections were of 0.9% saline in both.

Each experimental treatment was administered as two injections at 0930 h and 1330 h. The first was 0.25, 0.5, 0.75, or 1.0 mg/kg xylamidine or vehicle, and the second was 5.0 mg/kg 5-HT. All rats received all treatments. The order of treatments was determined by a 5 × 5 Latin square, and at least 2 drug-free days were allowed between experimental treatments.

After the second injection, the rats were weighed. Prewedged food hoppers (containing at least 150 g of food) were presented at 1430 h, and these were reweighed at 1530 h. The feeding period ended at 1830 h.

Results

Means for the 1st hour of food intake are shown in Figure 1. A one-way within-subjects analysis of variance yielded a significant main effect of treatment [$F(4,76) = 5.86, p < .0005$]. Post hoc analyses were carried out with Tukey's test. Pretreatment with 0.25 mg/kg of xylamidine did not attenuate the anorectic effect of 5-HT, since the food intakes of the vehicle + 5-HT (control) group and the 0.25 mg/kg xylamidine + 5-HT group were not significantly different. However, when pretreated with 0.5 mg/kg xylamidine, animals ate more than when given vehicle pretreatment ($p < .05$), and the size of the xylamidine pretreatment effect was even greater at 0.75 mg/kg and 1.0 mg/kg ($p < .01$ in both comparisons against the control treatment).

Figure 1. Dose-response curve for the effects of xylamidine on 5.0 mg/kg peripheral 5-HT-induced anorexia.

Discussion

These data support the hypothesis that xylamidine's antagonism of peripheral 5-HT-induced anorexia is dose-dependent. Although the differences between each dose of xylamidine did not reach statistical significance, the higher doses clearly blocked the 5-HT more effectively than the lower doses did. There was no significant difference between food intakes for control and for 0.25 mg/kg xylamidine, for example, but the difference was significant at the .05 level for 0.50 mg/kg xylamidine, and at the .01 level for 0.75 and 1.0 mg/kg xylamidine. On the basis of this data, it seems that xylamidine doses of 1.0 and 2.0 mg/kg would occur at the asymptote of the dose-response curve. Thus, at doses of 1.0 and 2.0 mg/kg, the failure of xylamidine to completely block 5.0 mg/kg peripheral 5-HT-induced anorexia (Edwards & Stevens, 1989) cannot be attributed to an insufficient dosage of xylamidine.

EXPERIMENT 2

Effect of Xylamidine on Drinking Behavior

The second possible alternative explanation for xylamidine's apparent failure to completely block peripheral 5-HT-induced anorexia is that xylamidine may have non-specific behavioral effects that may interact with this antagonism. One way of investigating this possibility is to test for the presence of a conditioned taste aversion (see below). However, since the methodology for such a test relies on the consumption of flavored water, it was necessary to check first whether xylamidine had any direct effect on intake of unflavored water.

Method

Subjects. Twelve male Wistar rats were used (286–328 g). Ambient conditions were as described above.

Drug. The drug used was xylamidine, prepared as described above. The dosage was 1 mg/kg.

Procedure. A within-subjects design was used, based on that of Deutsch and Hardy (1977). The testing of water intake took place over a period of 4 days. Initially, both standard rat chow and water were available ad lib. On Day 1, the rats were water-deprived at 1700 h. On Day 2, they were injected with the drug or vehicle at 1000 h, before being presented with preweighed water bottles at 1500 h. These bottles were removed and weighed at 1530 h, and the rats were once again given water ad lib. The procedure for Days 3 and 4 was the same as that for Days 1 and 2, except that the alternative injection was administered. Order of injection was counter-balanced, and water intakes were compared with a related-means *t* test.

Results and Discussion

The mean ($\pm$ SEM) water intake was 9.9 ± 0.4 g for the xylamidine group and 10.0 ± 0.5 g for the control group. There was no significant difference between the groups. It is clear that xylamidine had no effect on gross drinking behavior. Thus, the failure of xylamidine to completely block peripheral 5-HT-induced anorexia cannot be attributed to any effect that xylamidine might have on gross drinking behavior. Furthermore, the methodology pro-

posed to test for conditioned taste aversion, based on the intake of flavored water (Experiment 3), should not be confounded by any effect of xylamidine on food intake.

EXPERIMENT 3

Investigation of Whether Xylamidine Produces a Conditioned Taste Aversion

Although nonspecific behavioral effects have been ruled out as an explanation of 5-HT-induced anorexia (Pollock & Rowland, 1981), there is no evidence concerning the possibility that xylamidine's antagonism of peripheral 5-HT-induced anorexia may be confounded by such effects, so this possibility is investigated here. Any treatment that produces an anorectic effect may be doing so because it creates nauseous feelings in treated subjects. One way of detecting such an effect is to associate the treatment with a specific flavor of food or drink and thereby produce a conditioned taste aversion (CTA; Garcia, Kimeldorf, & Hunt, 1961; Nachman, 1963; Rozin, 1969). CTA has been investigated by adding a flavored substance to drinking water and pairing the putative nausea-inducing treatment with the ingestion of the flavored water. The presence of a CTA can then be determined some time later when the animal has recovered from the immediate effects of the treatment, using either a single-bottle or a two-bottle drinking test. In the former, the subject is allowed access to water containing the flavored substance, but in the latter, the flavored water is made available together with a second flavored water that has been presented previously without being paired with a nausea-inducing agent. Since the two-bottle test is thought to be the more sensitive and reliable measure of CTA (Deutsch & Hardy, 1977), this was the method chosen for Experiment 3.

Although we had confirmed that xylamidine has no direct effect on unflavored water intake (see above), the sensitivity of the CTA test was an unknown quantity in our hands, so we thought it prudent to check the test by using lithium chloride (LiCl) as the aversion treatment. Since this compound has been shown to produce a CTA in the less sensitive one-bottle paradigm at a dose as low as 7.5 mg/kg (Ervin & Teeter, 1986), the chosen dose of LiCl was 15 mg/kg, a dose that should produce an easily detectable CTA (Ervin & Teeter, 1986).

It was also decided that two intervals between xylamidine administration and ingestion of the flavored water should be tested. In one time condition, it was to be injected immediately after presentation of the paired flavor, and in the other condition, it was given 5 h before the flavored water. The treatment 5 h before injection should be more effective, since, as stated above, xylamidine has been shown to be more potent at this time than after shorter intervals.

Method

Subjects. For each of the four CTA tests, 24 male Wistar rats were used, with 12 assigned to the experimental condition and 12

to the control treatment. Ambient conditions were as described above.

Drugs. The drugs used were xylamidine, prepared as described above, and LiCl (Sigma). The LiCl was dissolved in distilled water and injected at a volume of 1.33 ml/kg. The dosages were 1.0 mg/kg (xylamidine) and 15 mg/kg (LiCl).

Procedure. For each CTA test, a between-subjects design was used. Initially, standard rat chow and water were available ad lib. On Day 1, the rats were water-deprived at 1700 h. On Day 2, the rats were presented with almond- or peppermint-flavored water for 30 min at 1500 h; then they were once again allowed water ad lib. The procedure for Days 3 and 4 was the same as that for Days 1 and 2, except that the alternative flavor was given and paired with an injection of either drug or vehicle. The precise timing of the injection varied with the drug used. On Day 5, they were again water-deprived at 1700 h. At 1500 h on Day 6, the rats were presented with preweighed bottles of water of both flavors for 30 min. Water intakes for the injection-paired flavor were recorded and compared for the drug and saline groups, using an independent *t* test. Injection-pairing of flavors and position of bottles were counterbalanced.

In the first test, the treatment was either LiCl or saline administered immediately after the flavor presentation period. In the second test, the treatment was either xylamidine or saline administered immediately after the flavor presentation period. In the third and fourth tests, the treatment was either xylamidine or saline administered 5 h before the flavor presentation period.

Results

Means of the intakes of the flavored water are illustrated in Figure 2. For the LiCl treatment, water intake was significantly depressed relative to the control group's intake ($t = 7.49, p < .0001$). When xylamidine was administered immediately following the flavor presentation period, water intake was nonsignificantly higher for the xylamidine group, relative to controls ($t = 1.92, .05 < p < .10$). This test was therefore repeated to test whether this apparent difference was reliable. In the repeat test, water intake was lower for the xylamidine group than for the controls, with no significant difference between the groups ($t = 1.49, p > .10$). When xylamidine was administered 5 h before the flavor presentation period, there was no significant difference between water intake for the two groups ($t < 1, p > .05$).

Discussion

On the basis of the data described here, there is no evidence to suggest that xylamidine can directly produce a CTA, either when it is administered immediately following presentation of the flavored water, or when it is administered 5 h before flavor administration. The latter finding is particularly important, for this is the interval used in the previous experiments in which xylamidine's effects on food intake were investigated. Furthermore, the failure of xylamidine to produce a CTA cannot be attributed to methodological problems, because the CTA produced by 15 mg/kg LiCl was clear and unambiguous. It can be concluded, therefore, that this experiment furnishes no evidence that the failure of xylamidine to completely block peripheral 5-HT-induced anorexia can be attributed to the production of a conditioned taste aversion.

Figure 2. Water intakes (in grams) for each CTA test. Xyl(a) = xylamidine injected immediately after flavor presentation. Xyl(b) = repeat of Xyl(a). Xyl(c) = xyl injected 5 h prior to flavor presentation. $p < .0001$. Center bars show SEM.

GENERAL DISCUSSION

Since xylamidine attenuates, but does not completely block, peripheral 5-HT-induced anorexia (Edwards & Stevens, 1989), it has been suggested that peripheral 5-HT-induced anorexia may be mediated by both 5-HT₂ and non-5-HT₂ receptor subtypes. However, this argument can only be sustained if it can be shown that the effects of xylamidine, like those of 5-HT (Pollock & Rowland, 1981), are not due to nonspecific effects. It has been demonstrated here that xylamidine does not affect drinking behavior or produce a conditioned taste aversion. Furthermore, it has been shown that the effects of xylamidine on peripheral 5-HT-induced anorexia is dose-dependent, and the dosages previously used (1.0 and 2.0 mg/kg) lie on the asymptote of the dose-response curve. Therefore, the previous failure of xylamidine to completely block 5-HT anorexia (Edwards & Stevens, 1989) cannot be attributed to insufficient dosages of xylamidine. Thus, taken together, the evidence presented in this paper supports the idea that peripheral 5-HT-induced anorexia is mediated by both 5-HT₂ and non-5-HT₂ receptor subtypes.

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