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Sweetness-Dependent Facilitation of Sucrose Drinking by Raclopride Is Unrelated to Calorie Content

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MUSCAT, R., T. KYPRIANOU, M. OSMAN, G. PHILLIPS AND P. WILLNER. *Sweetness-dependent facilitation of sucrose drinking by raclopride is unrelated to calorie content.* PHARMACOL BIOCHEM BEHAV **40**(2) 209–213, 1991.—Previous studies have reported that dopamine receptor antagonists increase the intake of solid or liquid diets containing high concentrations of sucrose. In Experiment 1, different groups of rats were trained in two-bottle tests (sweet solution vs. water), using three concentrations of either sucrose (0.7, 7 or 34%) or saccharin (0.02, 0.2 or 0.8%). Both sweeteners showed an inverted-U-shaped concentration-intake function. Raclopride increased intake of 34% sucrose, but not of 0.8% saccharin. In Experiment 2, raclopride had similar effects in three-bottle tests (all 3 concentrations available concurrently). However, whereas 34% was the most preferred sucrose solution, 0.2% saccharin was preferred to 0.8%. Thus, 0.8% saccharin differs from 34% sucrose in two ways, being not only noncaloric, but also aversive. In Experiment 3, 34% sucrose was rendered aversive by the addition of 0.08% quinine. Intake of this cocktail was not increased by raclopride. These results suggest that the difference between sucrose and saccharin in the effects of raclopride is related to the aversive properties of a concentrated solution of saccharin, rather than to its lack of calories.

Sucrose Saccharin Quinine Two-bottle test Three-bottle test Raclopride Domperidone Rats

UNDER most conditions dopamine receptor antagonists suppress rewarded behaviour. The hypothesis that this effect results from a decrease in the rewarding efficacy of the reinforcer maintaining the behaviour (18) receives support from a number of directions, including studies demonstrating that in the case of sweet rewards, the effects of dopamine receptor antagonists resemble those of a reduction in sweetness [e.g., (1, 4, 5, 15, 20)]. In apparent contrast to these findings is the observation that when very sweet rewards are used, dopamine receptor antagonists increase rewarded behaviour in rats. This effect has been observed both in operant behaviour maintained by a 95% sucrose pellet reinforcer (13,14) and in the consumption of very sweet (34% weight/volume) sucrose solution (9, 12, 13, 17).

There is some doubt as to the relationship between these paradoxical stimulant effects of dopamine antagonists and their putative effects on reward processes. An alternative account of the increased consumption of very sweet rewards derives from their high calorie content. Low doses of DA receptor antagonists can in some circumstances increase food intake (7,8). This effect is associated with a prolongation of eating (2) and may be specific to carbohydrates (14). Thus dopamine receptor antagonists could in principle increase sucrose consumption by delaying the onset of postprandial satiety. In the present study this hypothesis has been examined by comparing the effects of the selective D2/D3 receptor antagonist raclopride (3,16) on the consumption of sucrose and of the nonnutritive sweetener, saccharin.

Three experiments were performed. The first used two-bottle preference tests, which offered the choice between a single concentration of sucrose or saccharin, and water, at each of three concentrations of sucrose (0.7, 7 and 34%) and saccharin (0.02, 0.2 and 0.8%). Raclopride increased the intake of 34% sucrose but not of 0.8% saccharin. However, 34% sucrose and 0.8% saccharin differ not only in their calorie content, but also, potentially, in their palatability. This factor was examined in Experiment 2 by presenting a simultaneous choice of all three concentrations of sucrose or saccharin. In addition to examining the effects of raclopride in this paradigm, we also tested the effects of domperidone, a D2 antagonist that does not cross the blood-brain barrier (6), in order to ensure that the stimulant effect of raclopride on consumption of 34% sucrose was centrally mediated. In the three-bottle paradigm the most preferred solutions were the highest concentration of sucrose (34%), but the middle concentration of saccharin, which was preferred to the highest concentration (0.8%). Thus, 0.8% saccharin differs from 34% sucrose in two ways, being not only noncaloric, but also aversive. Experiment 3 investigated whether the aversive properties of 0.8% saccharin are responsible for the failure of raclopride to stimulate intake. This experiment again used three-bottle preference tests. One group of animals received three bottles of sucrose, as before, and a second group received 0.02% and 0.2% saccharin alongside 34% sucrose, in order that 34% sucrose should be the only source of calories. The third group also received 0.02% and 0.2% saccharin and 34% sucrose, but in this case, the 34% sucrose was rendered aversive by the addition of

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quinine. We reasoned that if the differential effect of raclopride on 34% sucrose and 0.8% saccharin results from the aversive properties of saccharin, then raclopride should fail to stimulate intake of the sucrose/quinine cocktail. However, if the relevant factor is calorie content, then the stimulant effect of raclopride on 34% sucrose intake should not be blocked by quinine adulteration.

METHOD

Subjects

Sixty male Lister hooded rats (NIMR, Mill Hill), weighing 275–325 g at the start, were used in Experiments 1 and 2, and a further 30 in Experiment 3. They were maintained on a 12:12-h light/dark cycle (lights on 0800 h) at a temperature of 22°C. The animals were singly housed and all testing was carried out in the home cage. Fluid intake tests were carried out following 23-h food and water deprivation; otherwise, food and water were freely available at all times.

Experiment 1

Six different groups of animals (n = 10) first received a continuous 48-h preexposure to one of three concentrations of either sucrose (0.7%, 7% and 34%, weight/volume) or saccharin (0.02%, 0.2% or 0.8% weight/volume), of commercial grade. During training, each animal was deprived for 23 h of food and water, then presented for 1 h with two preweighed bottles, one of which contained tap water, the other, sucrose or saccharin, at the same concentration as at preexposure. Fluids were presented in 300 ml white polythene bottles, with 5 cm ball tipped stainless steel tubes and close-fitting bungs. Consumption was measured by reweighing preweighed bottles at the end of the session. The two bottles were counterbalanced between animals across the left and right sides of the feeding compartment, and alternated in position from trial to trial. Tests were typically conducted on the Monday, Wednesday and/or Friday of each week. When baseline intakes had stabilized (9 sessions), all animals were administered each of four doses of raclopride (0, 100, 200 and 400 μg/kg), in a counterbalanced design with at least 2 drug-free days between tests.

Experiment 2

After completion of Experiment 1, in subsequent tests, the 30 animals that had received sucrose and water were now presented with a concurrent choice of all three sucrose concentrations (0.7, 7 and 34%), while the 30 animals that had been tested with saccharin and water were presented with a concurrent choice of all three saccharin concentrations (0.02, 0.2 and 0.8%). The positions of the three bottles (left/middle/right) were once again counterbalanced across animals and sessions. After intakes had stabilized (6 sessions) the effects of raclopride (0, 100, 200 and 400 μg/kg) were again determined, in a counterbalanced design with at least 2 drug-free days between tests. Subsequently, three-bottle testing continued, and after a drug-free period of 2 weeks, the experiment was repeated using domperidone (0, 2.5 and 5 mg/kg).

Experiment 3

Three groups of animals (n = 10) were trained in the three-bottle test, as described in Experiment 2. One group received 0.7%, 7% and 34% sucrose, as in Experiment 2; the other groups received 0.02% saccharin, 0.2% saccharin and 34% sucrose. After intakes had stabilized, these conditions were maintained during further testing in the sucrose/sucrose/sucrose and one of the saccharin/saccharin/sucrose groups. In the second saccharin/saccharin/sucrose group, the 34% sucrose was progressively adulterated, on successive tests, by the addition of increasing concentrations of quinine, starting at 0.01% and rising in 0.01% steps; between 1 and 3 tests were conducted at each concentration. The object of this procedure was to reach a concentration of quinine that produced a profile of three-bottle performance approximating that of the saccharin animals in Experiment 2 (i.e., a clear preference for 0.2% saccharin over the sucrose/quinine cocktail: see the Results section). This was achieved at a quinine concentration of 0.08%. The final conditions for this group thus consisted of a concurrent choice between 0.02% saccharin, 0.2% saccharin, and a cocktail of 34% sucrose and 0.08% quinine. After two further tests under these conditions, all animals were administered raclopride (0, 100, 200 and 400 μg/kg), in a counterbalanced design with at least 2 drug-free days between tests.

Drugs

Raclopride tartrate (Astra, Sodertalje) was dissolved in distilled water; domperidone (Janssen, Wantage) was dissolved in a minimal volume of glacial acetic acid and made up to volume with distilled water. Raclopride was administered 15 min and domperidone 45 min prior to testing. All injections were IP in a volume of 1 ml/kg.

Analysis

Results were analyzed by analysis of variance (ANOVA), supplemented by tests of simple main effects. Results analyzed were those obtained following drug or vehicle injections; baseline data were not included. In Experiment 1, sucrose and water intakes were analyzed together, and separate analyses were performed at each concentration of sucrose/saccharin; thus the ANOVA for each group had two within-subjects factors, fluid (sweet/water) and dose. In Experiment 2, data from sucrose and saccharin groups were analyzed separately; each ANOVA had two within-subjects factors, concentration and dose. In order to permit comparison across groups, all data were combined for analysis in Experiment 3. Thus, in addition to the two factors of Experiment 2, this ANOVA had an additional between-subjects factor of groups.

RESULTS

Experient 1

In the two-bottle tests, consumption of both sucrose and saccharin followed an inverted-U-shaped concentration-intake function, with maximal intake at the intermediate concentration (Fig. 1, top panels).

Raclopride (Fig. 2) dose-dependently decreased the intake of 0.07% sucrose, $F(3,54) = 111.1$, $p<0.001$, but had no effect on intake of 7% sucrose, $F(3,54) = 0.2$, N.S., and dose-dependently increased the intake of 34% sucrose, $F(3,54) = 5.5$, $p<0.01$, while reducing the intake of concurrently available water, $F(3,54) = 5.4$, $p<0.01$. In animals drinking saccharin, raclopride reduced intake at the two lower concentrations, $F(3,54) = 5.2$, $p<0.01$, and 4.1, $p<0.05$, respectively, but had no effect at the highest concentration (0.8%), $F(3,54) = 0.9$, N.S., and no significant effect on water intake in any group.

Experiment 2

In the three-bottle tests (Fig. 1, lower panels), rats showed a strong preference for the most concentrated (34%) sucrose solu-

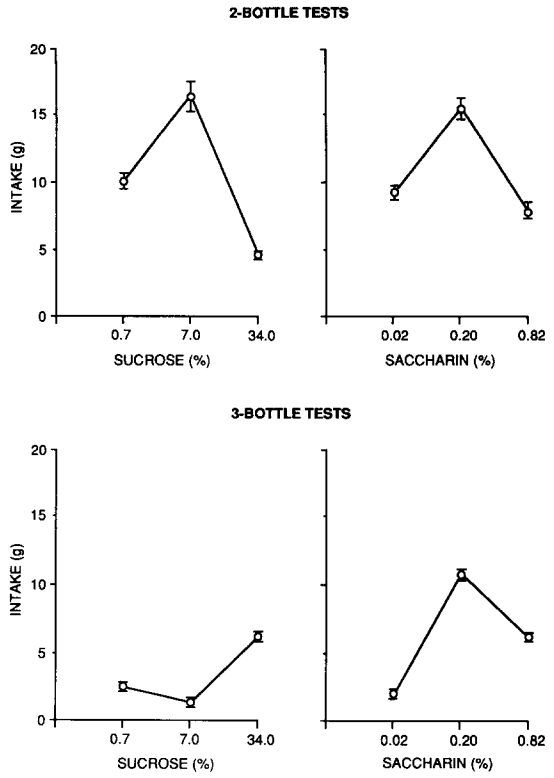

FIG. 1. Consumption of sucrose (left) and saccharin (right) in two-bottle tests (Experiment 1) in which different groups of animals were presented with a single sweet solution with water available as an alternative (above) and in three-bottle tests (Experiment 2) in which three concentrations of the sweet solution were presented concurrently (below). Values are means ± standard error.

tion, $F(2,58)=83.4$, $p<0.001$, but preferred the intermediate (0.2%) saccharin concentration [0.2% vs. 0.8%: $F(1,58)=24.9$, $p<0.001$].

Raclopride (Fig. 3) again decreased consumption of 0.7% sucrose but increased consumption of 34% sucrose, $F(3,87)=4.2$, $p<0.01$ and 32.0, $p<0.001$, respectively. As in Experiment 1, raclopride decreased consumption of 0.2% saccharin, $F(3,87)=7.1$, $p<0.001$, while not affecting consumption of 0.8% saccharin, $F(3,87)=0.6$, N.S. Domperidone decreased the consumption of both sucrose and saccharin; in particular, domperidone decreased the intake of 34% sucrose, $F(2,68)=6.1$, $p<0.01$, in contrast to the increases observed at this concentration with raclopride (Fig. 4).

Experiment 3

In Experiment 3, three-bottle performance in the two groups receiving unadulterated 34% sucrose was very comparable to that observed in Experiment 2: in both groups there was a strong preference for the 34% sucrose solution, and consumption of 34% sucrose was dose-dependently increased by raclopride (Fig. 5, left and middle panels). In animals receiving the 34% sucrose/quinine cocktail, 0.2% saccharin was the most preferred solution (Fig. 5, right panel). Consumption of the 34% sucrose/quinine cocktail was slightly lower than consumption of 34% sucrose by the other groups, but the differences were not significant, $F(1,27)=2.8$, N.S. In this group, raclopride dose-dependently decreased consumption of 0.2% saccharin, $F(3,81)=10.6$, $p<0.001$, but had no effect on consumption of the cocktail, $F(3,81)=0.2$, N.S.

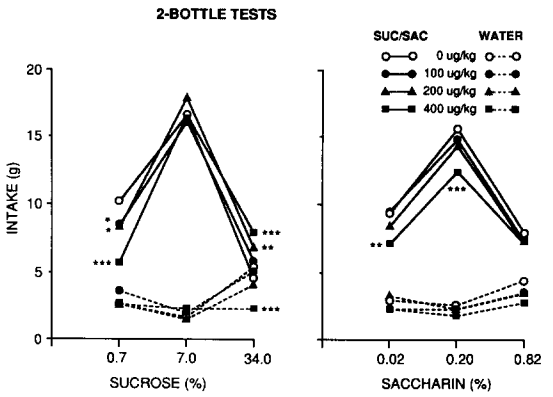

FIG. 2. Effects of raclopride on consumption of sucrose (left) or saccharin (right) and water in two-bottle tests (Experiment 1). Different groups of animals were tested at each concentration of sucrose/saccharin. Values are means; standard errors have been omitted for clarity. *$p<0.05$; **$p<0.01$; ***$p<0.001$, relative to vehicle treatment.

DISCUSSION

The present data confirm our earlier report that the effects of raclopride on sucrose consumption are concentration dependent (12): raclopride decreased the intake of 0.7% sucrose but increased the intake of 34% sucrose, in both two- and three-bottle tests. Similar effects of raclopride are also observed in single-bottle tests (unpublished observations). Although the three-bottle data of Experiment 2 were obtained in animals with prior experience of both two-bottle testing and raclopride administration, the results were replicated in Experiment 3; the same phenomena were also observed in both experienced and naive animals in our earlier study (12). Increases in preference for 34% sucrose over water have also been reported with pimozide, sulpiride and SCH-23390 (9, 13, 17). The failure of domperidone, a D2 antagonist which does not cross the blood-brain barrier (6), to increase the consumption of 34% sucrose confirms that this

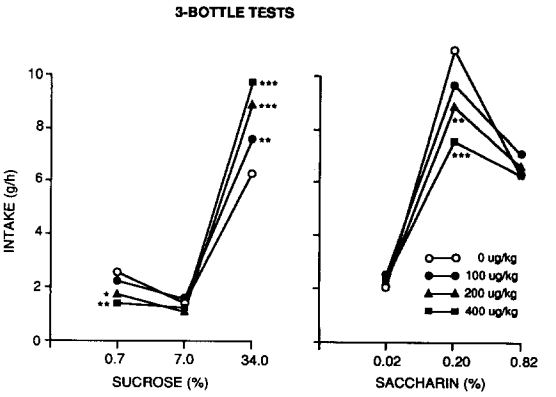

FIG. 3. Effects of raclopride on consumption of sucrose (left) or saccharin (right) in three-bottle choice tests (Experiment 2). Values are means; standard errors have been omitted for clarity. *$p<0.05$; **$p<0.01$; ***$p<0.001$, relative to vehicle treatment.

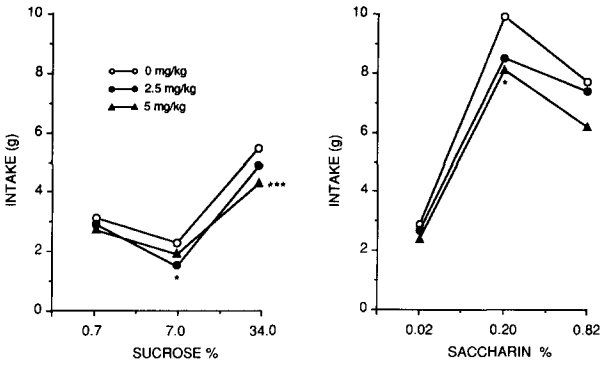

FIG. 4. Effects of domperidone on consumption of sucrose (left) or saccharin (right) in three-bottle choice tests (Experiment 2). Values are means; standard errors have been omitted for clarity. $*p<0.05$; $***p<0.001$, relative to vehicle treatment.

effect is of central origin.

Although the animals were deprived of both food and water [a procedure adopted to maintain consistency with our earlier study (12)], it is clear from the two-bottle data (Experiment 1) that water balance is not a factor in the stimulant effect of raclopride on intake of 34% sucrose. Thus, despite the osmotic load incurred by ingesting a concentrated solution of sucrose, the increase in sucrose intake following raclopride treatment was accompanied by a commensurate decrease in water intake, such that total fluid intakes after vehicle or 400 μg/kg raclopride were almost identical (Fig. 1). Further evidence against an involvement of osmotic factors are the lack of effect of raclopride on intake of the sucrose/quinine cocktail, which provided a comparable osmotic load to 34% sucrose alone, but was not increased by raclopride (Experiment 3); and the fact that raclopride, as well as pimozide, have also been shown to stimulate the intake of very sweet solid food, in animals that were not water deprived (10,11).

In two-bottle tests (Experiment 1), sucrose and saccharin produced comparable inverted-U-shaped concentration-intake functions. The lack of effect of raclopride at the highest saccharin concentration, in contrast to the stimulation of intake observed at the highest sucrose concentration, is compatible with the hypothesis that the stimulation of sucrose intake is related to calorie content. However, data from the three-bottle tests (Experiment 2) suggest an alternative explanation. The three-bottle test reveals that, while the intermediate (7%) sucrose solution supports the highest intake, the highest (34%) concentration is most preferred. By contrast, the intermediate (0.2%) concentration of saccharin not only supported the highest levels of consumption in the two-bottle test, but was also the most preferred in the three-bottle test. It follows that the highest concentrations of sucrose (34%) and saccharin (0.8%) differ in two ways: in addition to being calorie-free, 0.8% saccharin also has aversive properties. This presumably reflects the emergent bitter taste of saccharin at high concentrations.

Experiment 3 was carried out to investigate which of these dimensions is responsible for the difference in the effect of raclopride on consumption of 34% sucrose and 0.8% saccharin. In order to ensure that 34% sucrose provided the only source of calories, one of the control groups was presented with a choice between low (0.02%) and intermediate (0.2%) concentrations of saccharin and 34% sucrose; the behaviour of this group was very comparable to that of the sucrose-sucrose-sucrose controls, and raclopride increased intake of 34% sucrose in both groups. In the Experimental group 34% sucrose was adulterated with quinine, which caused a small and nonsignificant decrease in sucrose consumption, but reversed the preference relationship between 34% sucrose and 0.8% saccharin. Under these conditions, raclopride had no effect on consumption of 34% sucrose. As the calorie content of the sucrose/quinine cocktail was identical to that of the unadulterated sucrose, it follows that calorie content cannot be the crucial factor underlying the stimulation of 34% sucrose intake by raclopride.

Evidence from other studies supports the conclusion that the stimulation of 34% sucrose intake by neuroleptics is related to sweetness rather than calorie content. Firstly, raclopride has been shown to increase rates of continuously reinforced operant responding maintained by solid 95% sucrose pellets, while not affecting responding maintained by standard (10% sucrose) pellets of an almost identical calorie content (11). Secondly, if the effect were calorie-related, then it would not be apparent until

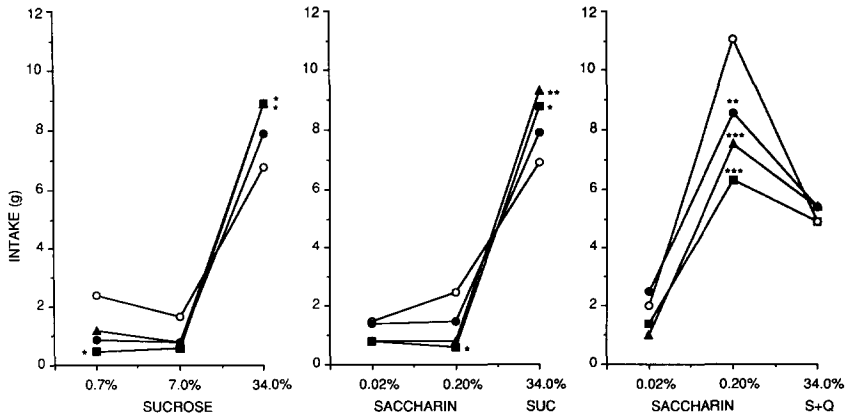

FIG. 5. Effects of raclopride on consumption of sucrose and saccharin solutions in three-bottle choice tests (Experiment 3). Different groups received three concentrations of sucrose (left), two concentrations of saccharin and 34% sucrose (center), or two concentrations of saccharin and a 34% sucrose/0.08% quinine (S + Q) cocktail (right). Values are means; standard errors have been omitted for clarity. $*p<0.05$; $**p<0.01$; $***p<0.001$, relative to vehicle treatment.

some way into the session, since if calories are to be effective, they must first be absorbed. However, stimulatory effects of raclopride are apparent within the first 5 min of the session, and do not increase in size thereafter (11,12).

To summarize, the stimulant effect of raclopride on intake of 34% sucrose cannot be expained by postingestional factors relating either to water balance or to calorie content. It follows that this effect is more likely to reflect a change in palatability of the sucrose. The palatability of any taste is determined jointly by its appetitive and aversive properties, and in principle, a change in palatability could result from changes in either of these components. However, there is no evidence that this effect of raclopride involves changes in the aversive properties of 34% sucrose. For one thing, there is no evidence that 34% sucrose has any aversive properties: in a choice between different sucrose concentrations (the three-bottle test), 34% was the most preferred concentration. Furthermore, if raclopride had an antiaversive action, it should increase the intake of 0.8% saccharin and the sucrose/quinine cocktail; it did not.

By exclusion, the most promising hypothesis is that raclopride acted in this study by changing the appetitive (rewarding) properties of 34% sucrose. In fact, all the effects of raclopride observed in the present study are compatible with the hypothesis that dopamine receptor antagonists reduce the rewarding properties of sweet solutions (18,19). In the case of saccharin, the intermediate sucrose concentration is both most rewarding (three-bottle test) and most consumed (two-bottle test), so a decrease in reward would cause a decrease in consumption. The same is true for dilute solutions of sucrose, on the ascending limb of the concentration-intake function. The anomaly at 34% sucrose arises because at high concentrations there is a disjunction between reward and consumption: relative to 7% sucrose, 34% sucrose is preferred (three-bottle test) but underconsumed (two-bottle test). The reason for this discrepancy remains to be established, but the consequence is clear: at high sucrose concentrations, the quantity consumed is not monotonically related to reward. On the contrary, as sucrose concentration increases, reward (as measured by preference) increases, but consumption decreases. Paradoxically, therefore, a decrease in reward might be reflected in an increase in consumption; and this is what is observed following the administration of dopamine receptor antagonists.

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