Pharmacology Biochemistry & Behavior, Vol. 33, pp. 21–26. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Stimulation and Inhibition of Food Intake by the Selective Dopamine D_2 Agonist, N-0437: A Meal Pattern Analysis

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Received 20 July 1988

CLIFTON, P. G., I. N. RUSK AND S. J. COOPER *Stimulation and inhibition of food intake by the selective dopamine D_2 agonist, N-0437: A meal pattern analysis*. PHARMACOL BIOCHEM BEHAV **33**(1) 21–26, 1989.—Feeding and drinking responses were recorded in home-caged rats over 24-hour periods, and the data were analysed in terms of meal patterns. The highly selective dopamine D_2 receptor agonist, N-0437, was injected IP at the start of the night phase. At the smallest dose (0.3 mg/kg), N-0437 increased food intake as a result of increases in meal size and duration. The rate of feeding during meals was not increased. This effect lasted 1–2 hours in the night phase. At higher doses (1.0 and 3.0 mg/kg), N-0437 inhibited food intake for 3–6 hours. The inhibition was due to a substantial reduction in meal size, with no change in meal frequency. Rate of feeding during meals tended to be reduced. A biphasic dose-effect relationship was not apparent in the drinking data, and there was not a significant effect of N-0437 on drinking responses. The meal pattern analysis results indicate the stimulation of D_2 receptors produces increases and decreases in food intake and meal size. These results are discussed in terms of dose-dependent stimulation of presynaptic (autoreceptor) and posysynaptic D_2 receptors, and the relationship to satiety within meals.

Dopamine D_2 receptor Meal pattern analysis N-0437 Satiety

IT is generally recognised that monoamine neurotransmitters exert effects through multiple receptor subtypes. The distinction proposed by Kebabian and Calne (12) for central dopamine receptors, dividing them into D_1 and D_2 subtypes, is widely accepted. The availability of novel drugs which are selective for receptor subtypes (18) has made progress possible in linking dopamine receptor subtypes to the mediation of behavioural responses. Beaulieu and colleagues (3) synthesised and tested a series of N,N-disubstituted 2-aminotetralins, and reported on several compounds which displayed selective D_2 agonist activity, with little or no D_1 agonist activity. Of these compounds, N-0437 [2-(N-propyl-N-2-thienylethylamino)-5 hydroxytetralin], proved to be the most potent (3,20). [^{3}H]N-0437 has been used to label D_2 receptors in calf and rat brain (19,21). Consequently, we selected N-0437 as an appropriate pharmacological tool to investigate the involvement of dopamine D_2 receptors in the control of ingestional responses.

Studies with centrally administered dopamine (13,14) and systemically administered dopamine agonists, e.g., (1, 2, 6, 11), indicate that agonist activity at dopamine receptors reduces food consumption in rats. Using N-0437, we have recently shown that stimulation of D_2 receptors is sufficient to reduce food intake in rats and mice (17). The aim of the present experiment was to extend our original observations on N-0437, and to study changes in feeding behaviour in detail, using meal pattern analysis in freely-feeding animals (4, 5, 8).

As Blundell has suggested (4), measurement of feeding patterns in free-feeding rats follows from an attempt to improve the ecological validity of procedures designed to investigate the controls of feeding behaviour. Meal pattern analysis permits more detailed and specific characterizations of the effects of drug treatments on feeding responses (4). The present study is the first to investigate the effects of a selective D_2 agonist on 24-hr feeding and drinking patterns. Most interestingly, the results revealed both an increase in food intake (at a small dose), and a reduction (at higher doses) following N-0437 administration. The adjustments to meal size and patterning which underlie these changes in food intake are described.

METHOD

Animals

Eight male hooded Lister rats from the University of Sussex

colony weighing between 271 and 319 g at the beginning of the experiment were housed singly in cages (45 × 30 × 30 cm). There was a small (15 × 10 × 8 cm) open top nest box in one corner of the cage. The cages were held in a single experimental room, maintained at 24°C, in visual but not auditory isolation from each other. The room was maintained on a 12:12 hour L/D cycle with lights off at 11.30 hr. A single 25-W red bulb provided minimal illumination during the dark phase. Daily handling, weighing, drug administration and refilling of food and water containers was carried in the final 30 minutes of the light period. The animals were habituated to the room and food source for 8 days before the experiment began.

Apparatus

Food (45 mg Campden pellets) and water were freely available throughout the experiment. Intake was recorded using a microprocessor based system (8). A single pellet was always available in a small hopper attached to one wall of the cage. When this pellet was consumed it was replaced within a second and the time logged. Only very rarely did the animals drop pellets below the perforated cage floor and no hoarding was possible in these cages. Therefore, pellet removals were an accurate record of food intake. Water was dispensed from a nozzle situated 15 cm from the food hopper. The change in capacitance produced by licking the nozzle activated a peristaltic pump that provided water; again the time at which the pump was activated were recorded automatically.

Procedure

N-0437 (5,6,7,8-tetrahydro-6-propyl[2-(2-thienyl)ethyl] amino]-1-naphthalenol) hydrochloride, a highly selective D_2 agonist (3,20), was kindly supplied by Nelson Research, Irvine, CA. It was dissolved in distilled water and injected IP at doses of 0, 0.3, 1 and 3 mg/kg. The drug was administered immediately after daily weighing and 5 minutes before the beginning of the dark phase. Each animal received each dose with order counterbalanced over the eight subjects. Drug-free days alternated with those on which the drug was given.

Analysis

Food and water intake patterns were analysed in two ways. First, the numbers of feeding and drinking responses occurring in a given time were calculated. These numbers are either presented directly (e.g., Fig. 1) or further transformed using the cumulative sum technique (24). This technique, which is widely used in quality assurance, shows changes in the rate of feeding as changes in slope. The plot is simply produced by summing the mean rate of feeding over relatively short time periods (here 30 minutes) and subtracting, from each value, either the overall mean or some value close to it. The result is that a constant rate of feeding equal to the mean is shown as horizontal line, and rates either above or below the mean are shown as lines of positive or negative slope. Such plots have the property that small changes in overall rate of feeding quickly become apparent making it, for example, much easier to determine the period of action of a drug. This is true even when the variance in the data is relatively high. This is inevitably the case with a behaviour like feeding that only occurs periodically.

The second form of analysis examined the patterning of meals into which feeding is structured. The first problem in a meal analysis is to define a suitable criterion for meal termination. We used the log-survivorship technique in which the distribution of interpellet intervals is cumulated backwards and plotted on a logarithmic y-axis. Such plots have the property that the probability of an interval ending is proportional to the slope of the curve at that point. For our data the curves are well approximated by two negative exponentials, one of large negative slope (intrameal intervals) and a second of lower slope (intermeal intervals). A criterion of 2 minutes efficiently separates these two distributions (8). After this criterion was applied to the data, meal size was defined as the number of pellets eaten after a first interpellet interval exceeding 2 minutes and before the next interpellet interval greater than this value. Meal duration was defined as the time between taking the first and last pellets of a meal and feeding rate was calculated by dividing the number of pellets taken in a meal by its duration. The intermeal interval was defined as the time between taking the last pellet of one meal to taking the first pellet of the next meal.

Data for one individual at 1 mg/kg and another at 3 mg/kg were not analysed because of feeder faults on either that day or the previous drug-free day. As a result the analyses of variance that are reported for the within-subject design contained two fewer degrees of freedom than would be expected from the experimental design; the analyses themselves were performed using the missing values procedure of GENSTAT.

RESULTS

Daily Intake Patterns

In the initial analysis, feeding and drinking records were summed into 3-hour blocks. A dose of either 1 or 3 mg/kg N-0437 produced a clear anorexia over the first three hours which extended into the second three-hour block at the higher dose (Fig. 1). The lowest dose of 0.3 mg/kg produced an increase in feeding in the first 3-hour time block and a depression in the second. Intake patterns over the remainder of the 24-hour period showed the typical late night increase in feeding followed by a low rate of feeding throughout the day; this pattern was similar after all four treatments. Analysis of variance of the entire data set for feeding produced significant main effects of drug dose and time with, in addition, a significant interaction, $F(3,201) = 3.09$, $p<0.03$; $F(7,201) = 31.02$, $p<0.001$; $F(21,201) = 2.37$, $p<0.001$. Paired t-tests between the saline and drug treatments in the first time period showed a significant increase with the low dose, $t(7) = 3.66$, $p<0.01$, and a significant decrease with the high dose, $t(6) = 2.91$, $p<0.0326$. The difference between the control and 1 mg/kg dose was nonsignificant, $t(7) = 1.33$, $p = 0.22$.

Drinking patterns showed a similar distribution over the entire experimental period to that for feeding. Intake was concentrated into the early and late parts of the dark period. However, the effects of drug dose in the early part of the day showed a rather different pattern and proved to be less consistent across individuals. The two lower doses (0.03 and 1.0 mg/kg) produced a slight depression in drinking, whereas the highest dose (3.0 mg/kg) enhanced drinking. However, although these changes were marked in some individuals, an analysis of variance produced only a significant effect of time, $F(7,201) = 31.05$, $p<0.001$. The drug effect was nonsignificant. This remained true even when the analysis was restricted to the first three hours' data.

It is clear from Fig. 2, in which food intake is plotted as a cumulative sum (see the Method Section), that the facilitatory effect of 0.3 mg/kg N-0437 lasts for no more than 2 hours and is followed by a brief period in which food intake is reduced below control levels. The inhibitory effect of 1 mg/kg lasted for about two hours but in this case there is little evidence of a subsequent compensatory increase in food intake. The highest dose (3 mg/kg) produces both a more profound and longer lasting reduction in

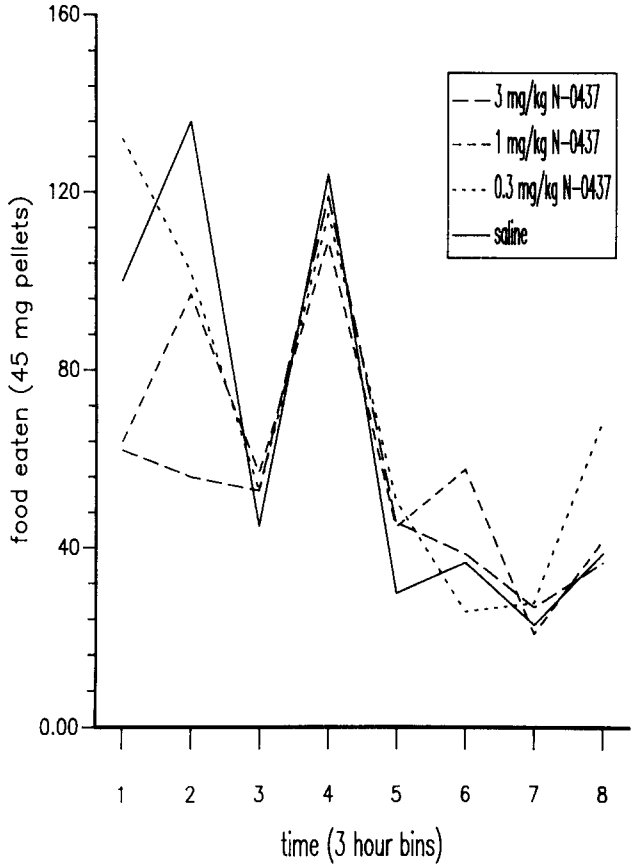

FIG. 1. Effects of N-0437 on food intake over a twenty-four-hour period. The mean number of pellets consumed by each animal in three-hour time blocks is plotted against time. The first four points show consumption during the night period and the last four points show consumption during the day period. The standard error for the difference between means for the time × day stratum in the ANOVA was 16.67 pellets.

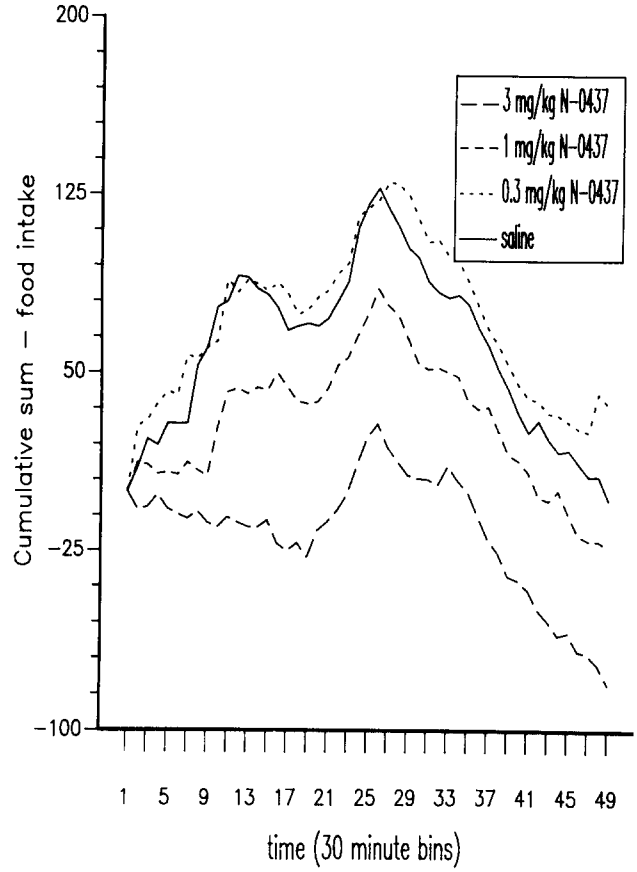

FIG. 2. A Cusum plot for food intake using 30-minute feeding totals. The text describes the method used to produce this plot. Feeding at a mean rate of 10 pellets in each time bin (here 30 minutes) gives a horizontal line. Feeding rates above this value give a line of positive slope and below this value give a line of negative slope.

food intake; again there is no evidence for a later compensatory response in the data.

Meal Patterns

All animals, regardless of drug treatment, showed a clear pattern of eating in meals. This resulted in a log-survivorship curve for interpellet intervals that was approximated by the sum of two negative exponentials (8). The graph changed slope most rapidly at intervals of about 90 seconds and 2 minutes was chosen as a criterion that would clearly distinguish within meal from between meal intervals. An analysis of meal patterns in which the data for the dark and light periods were separated showed no effects of drug treatment on the light period which did not begin until 12 hours after drug treatment. Meals during this period were, as in previous studies using this apparatus, of about the same mean size as those in the dark period but were much less frequent.

Table 1 shows the meal parameters for each drug dosage during the dark period. Drug treatment had a significant effect on meal size, $F(3,26) = 10.18$, $p<0.001$. Meal size was clearly and significantly reduced by the highest dose of N-0437, although the increase in meal size after the lowest dose did not achieve significance in this 12-hour period. Dose had no significant effect on either the number of meals or meal frequency, $F(3,26) = 2.31$, 1.83 respectively, NS. Indeed the mean frequency was increased rather than decreased at the highest dose. The significant change in meal size was paralleled by significant changes in meal duration, $F(3,26) = 5.85$, $p = 0.003$. The increase in meal duration with the lowest drug dose and the decrease with the highest were both significant.

Feeding Rate

Feeding rate within meals was reduced by all doses of N-0437, although this did not give rise to a significant effect in the analysis of variance of the individual means, $F(3,26) = 1.79$, $p = 0.17$; this analysis was based on data for meals in the whole of the 12-hour dark period. However, the distributions of interpellet intervals (IPI) in the three hours following drug admnistration showed very marked changes (Fig. 3). The lowest dose of N-0437 increased the median IPI from 7 to 8.5 seconds. Although the median showed further increases at the two higher doses (10.5 and 13 seconds, respectively), these changes were associated with complex changes in the form of the distribution with both shorter and longer intervals becoming relatively more common (Fig. 3). This is especially obvious for the 3 mg/kg dose of N-0437 (lower RH panel, Fig. 3), in which the distribution shows an early peak at 2–3 sec and is then relatively flat over longer IPI's (e.g., 7–8 sec)

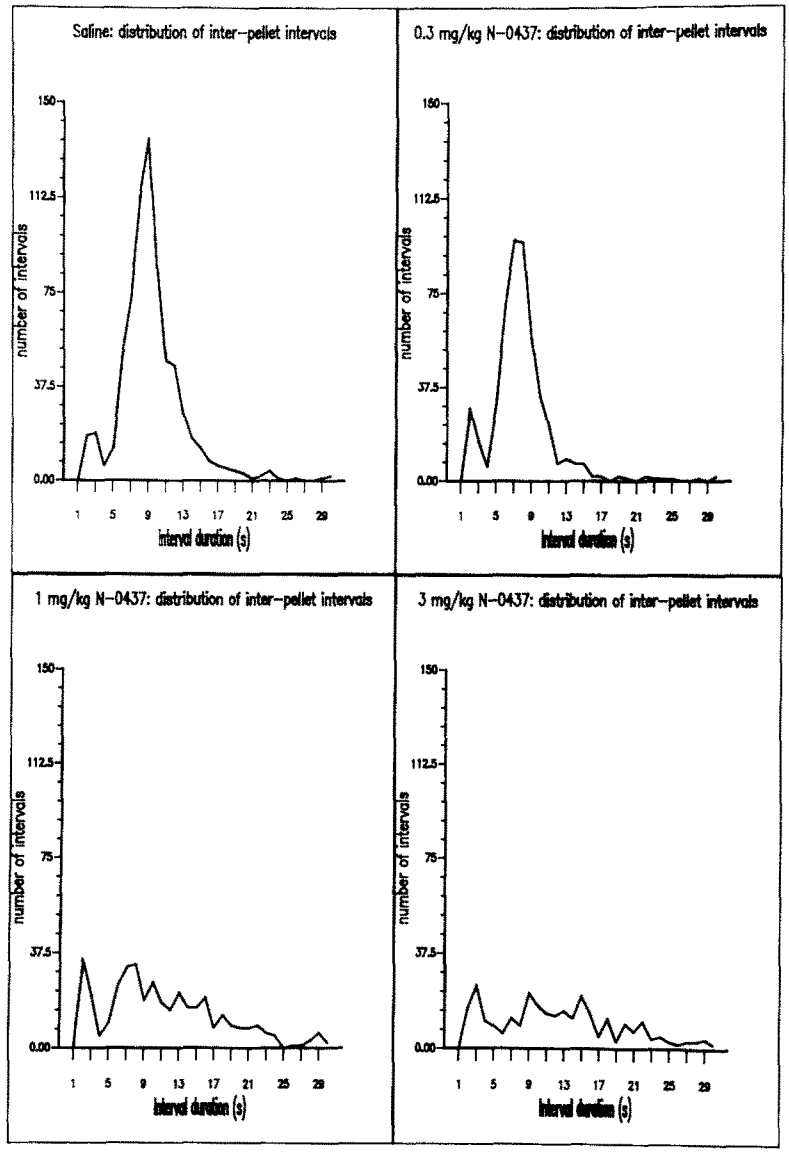

FIG. 3. Frequency distribution of interpellet intervals during the 3 hours following treatment with saline or N-0437. The intervals were classified into one of 30 bins each of which was 1 second in width.

which are typical of control animals taking and eating single pellets. Direct observation of the animals indicated that, at the higher doses, the animals, as well as eating many fewer pellets, also tended to pick up 2 pellets and then chew them both rather than, as is normally the case, taking single pellets. The resulting distribution therefore included fewer IPI's and lacked the characteristic peak at 7–8 sec. As mentioned in the Method section, very few pellets were dropped by the animals, so it is clear that these short interpellet intervals were not due to the animals dropping one pellet through the cage floor and immediately picking up a second.

DISCUSSION

The meal pattern analysis showed that N-0437, a selective D_2 agonist, produced biphasic changes in food intake during the 12-hour night-period following its administration. The changes in food intake were due to effects upon meal size and duration, but not on meal frequency. For convenience, the increase and decrease in food consumption produced by N-0437 will be considered separately.

At 0.3 mg/kg, N-0437 increased meal size and duration, with a slight (nonsignificant) reduction in feeding rate. This is an important observation since it indicates that D_2 receptor stimulation can lead to enhanced feeding responses. Since, in small doses, N-0437 preferentially activates dopamine D_2 autoreceptors (20,22), it seems probable that the increase in meal size was mediated presynaptically by dopamine autoreceptors. In support of this possibility, N-0437 at 0.3 mg/kg induces yawning and stretching, and produces hypolocomotion (Rusk and Cooper, unpublished data). Each of these effects is consistent with dopamine autoreceptor activation (10). Biochemical evidence indicates that N-0437 in small doses inhibits the release of dopamine through its

TABLE 1

NIGHT-TIME MEAL PARAMETERS

	Drug Dose (mg/kg)				
	Veh	0.3	1.0	3.0	SED	S
Total pellets	424	435	350	303	29.3	*
Number of meals	15.0	13.6	13.6	17.1	1.55	NS
Meal size	29.7	33.0	26.5	17.9	2.87	*
Meal duration	247	296	237	183	26.9	*
Feeding rate within meals	0.121	0.112	0.113	0.099	0.007	NS
Intermeal interval	52.3	57.1	52.8	42.9	6.25	NS

Total pellets is shown as the number of 45 pellets consumed during the dark period; meal size is also expressed in pellets. Meal duration is given in seconds and intermeal interval is shown in minutes. Feeding rate is shown as the number of pellets eaten per second during the meal. The penultimate column shows the standard error of the difference between means in the analysis of variance for that variable. The final column indicates whether the F-ratio from the analysis was significant ($p<0.05$).

activation of D_2 autoreceptors (20,22). We propose, therefore, that in free-feeding rats stimulation of dopamine presynaptic receptors, with consequent inhibition of dopamine release, is sufficient to increase meal size and meal duration.

Recently, N-0437 has been resolved into its (+) and (−) enantiomers (23). Both (+) and (−) N-0437 stimulate presynaptic dopamine receptors; postsynaptically (−) N-0437 is a D_2 agonist while (+) N-0437 is a weak antagonist (23). We predict, therefore, that at autoreceptor-stimulating doses, both enantiomers of N-0437 would increase meal size and duration. One of the most selective presynaptic dopamine agonists currently available is N,N-di-n-propyl-7-hydroxy-2-aminotetralin (DP-7-ATN) (16), and we expect that it, too, will stimulate feeding responses in free-feeding rats. Experiments are underway to investigate these possibilities.

At higher doses, N-0437 is a very potent agonist at postsynaptic D_2 receptors (20,22). Our previous report indicated that at 1.0 and 3.0 mg/kg, N-0437 significantly reduced food consumption in rats (17). The present data confirm and extend these results and show that at these doses N-0437 reduced meal size and meal duration, without significantly affecting meal frequency. Thus, selective stimulation of postsynaptic D_2 receptors is sufficient to reduce meal size in free-feeding rats.

Taken together, the present data are consistent with the notion that dopamine activity at postsynaptic D_2 receptors has an inhibitory function in determining meal size in free-feeding rats. Stimulation of presynaptic dopamine receptors which results in a reduction in dopamine release, and consequently a reduction in postsynaptic receptor, would increase meal size. Conversely, direct stimulation of postsynaptic D_2 receptors decreases meal size. Evidence which is consistent with this interpretation comes from a study with gastric sham-feeding rats, which show pronounced satiety deficits (25). We found that N-0437 did not affect sucrose sham-feeding, even though sucrose real-feeding was significantly reduced at 1.0 and 3.0 mg/kg (9). In the absence of satiety cues, N-0437 was ineffective on sucrose consumption. Thus, postsynaptic D_2 receptor activation may interact with satiety signals to limit meal size in free-feeding animals. In support of this view, N-0437 at higher doses slowed the rate of feeding within meals. According to Le Magnen (15), eating rate declines within meals, as satiety develops. Furthermore, a recent biochemical study provided strong evidence that increased metabolism of dopamine in the brain is associated with satiety, as distinct from the act of feeding (7).

It is interesting to note that the pattern of changes which occurred following N-0437 was not repeated for pre- and postsynaptic D_2 receptors may be limited to feeding responses, and cannot be extended to drinking behaviour. This would support an interpretation in terms of specific interactions of dopamine with the systems controlling food intake rather than more general arousal effects.

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