A behavioural profile of fluoxetine-induced anorexia

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Abstract. Fluoxetine is a specific and long-lasting inhibitor of serotonin reuptake. In free-feeding rats a dose of 10 mg/kg reduced meal size but had no significant effect on meal frequency. Feeding rate during meals was also reduced. Direct observation of behaviour associated with eating suggested that fluoxetine did not act by enhancing sleep or other behaviour patterns that interfere with eating, although the transition from feeding to sleep occurred more rapidly after drug treatment. Enhancement of satiety or interference with the sustaining of meals by fluoxetine would be consistent with these data. Rebound feeding after anorexia was not observed in either the meal pattern study or in a separate experiment using schedule fed animals. There was also no clear development of tolerance to the anorectic effect of fluoxetine, and we discuss possible reasons for an association of these two properties.

Key words. Fluoxetine – 5HT reuptake inhibitor – Anorectic drug – Meal patterns – Satiety sequence – Tolerance

There has been some discussion about the extent to which the anorectic actions of drugs enhancing serotonergic function can be differentially attributed to the enhancement of 5-HT release or inhibition of 5-HT reuptake. Fenfluramine, a well characterised anorectic agent (Rowland and Carlton 1986), has both of these effects. Garattini et al. (1986) point out that some potent inhibitors of 5-HT reuptake (e.g., indalpine) are only weakly anorectic, whereas direct 5-HT agonists generally do produce a marked anorexia (e.g., quipazine, MCPP). However, this distinction does not hold in all cases, since other 5-HT reuptake inhibitors, of which fluoxetine and CGS 10686B are examples, are potent anorectic agents (Goudie et al. 1976; Kim and Wurtman 1988).

Here we apply a series of behavioural techniques to the study of fluoxetine anorexia, both to provide a comparison with agents having less specific effects on serotonergic systems and to suggest the behavioural mechanisms producing anorexia in this case. The analysis of the meal patterns of free-feeding animals, as in experiment 1, provides a sensitive assay of both the magnitude and duration of anorexia. In addition, intake may potentially be reduced in many ways (reduction in meal size, reduction in meal frequency etc.) and the particular change can both suggest the mechanisms through which a particular drug acts, and also whether different drugs act through a common route.

Direct observation of the sequence of behaviour occurring after food consumption (the "behavioural satiety sequence") has been used to discriminate between anorexia produced by enhancement of satiety, or induction of sickness, respectively (Antin et al. 1976). It also has the advantage, especially when behaviour during periods with and without food consumption is studied, of being sensitive to sleep-inducing and other general changes in behaviour that may indirectly affect feeding patterns. Experiment 2 describes such a study of fluoxetine-induced anorexia.

Fluoxetine is an interesting drug in other respects; tolerance develops rapidly to most anorectic drugs, whereas this effect is not seen with fluoxetine (Rowland et al. 1982); this finding is replicated in experiment 3a. A final experiment examines the extent of rebound feeding after fluoxetine-induced anorexia and a possible relationship to the absence of tolerance to the anorectic effect of this drug is postulated.

Materials and methods

Animals. Male hooded Lister rats from the University of Sussex colony were used in all the experiments reported here. They weighed between 200 and 325 g at the beginning of the individual experiments and new naive subjects were used on each occasion. They were housed singly, with free access to pelleted chow (BOCM) and water. The experimental rooms were maintained at 23-24°C on a 12:12 h L/D cycle in which a 25 W red bulb provided minimal illumination during the dark period.

Drugs. Fluoxetine hydrochloride (Lilly 110140), kindly supplied by M.J. Schmidt of Lilly Research Laboratories, was dissolved in warm normal saline (10 mg/ml) and injected at a dose of 10 mg/ml. Injections were SC into the nape of the neck and the animals were given at least two injections of saline of similar volume (1 ml/kg) to habituate them to the general procedure before an experiment was begun.

Procedures. In experiment 1 eight rats, weighing between 230 and 265 g at the beginning of the experiment, were housed singly in large 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 on a reversed L/D cycle (lights off: 17.30 h), in visual
but not auditory isolation from each other. Daily handling, weighing, drug administration and refilling of food and water containers was carried out in the final 30 min of the light period. The animals were habituated to the room and this source of food for 8 days before the experiment began.

Food (45 mg Campden pellets) and water were freely available throughout the experiment. Intake was recorded using a microprocessor based system (Clifton 1987). 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 for 0.2 s; again, the times at which the pump was activated were recorded automatically. Each pump activation delivered about 0.02 ml water.

Fluoxetine was administered immediately after daily weighing and 15–20 min before the beginning of the dark phase. All animals were given saline injections on days 1 and 4 of the experiment and fluoxetine on days 2 and 3. This design was chosen so that a direct comparison could be made with animals in experiment 3a in which fluoxetine was also given daily. The data were initially analysed by examining food intake in 6-h bins. Meal patterns of individual animals were produced after a log-survivorship analysis of the intervals between pellets. In such a plot (e.g., Fig. 3a) the probability of an interval ending is proportional to the slope at that point. Thus the probability of an inter-pellet interval ending (i.e., another pellet being taken) is initially high but, after 2 min or so, rapidly declines. This point provides a suitable criterion for the separation of within- from between-meal intervals. Clifton (1987) discusses a quantitative method, which was followed in this case, by which this criterion may be chosen. Although it is possible to choose criteria for each animal in the study, this makes the interpretation of derived parameters (such as meal size and feeding rate) more difficult, and instead we used a single criterion for all individuals. The chosen criterion was 2 min but all the calculations were repeated with a much longer criterion of 10 min.

After this criterion was applied to the data, meal size was defined as the number of pellets eaten after a first inter-pellet interval exceeding 2 min and before the next inter-pellet 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 inter-meal interval was defined as the time between taking the last pellet of one meal to taking the first pellet of the next meal. Three individual records were discarded (of the 32 that were collected) because of minor equipment faults. The analyses of variances that are reported used the missing values procedure of GENSTAT statistical package and therefore have three fewer degrees of freedom in the numerator than would be expected from the experimental design.

In the second experiment 12 rats weighing between 240 and 325 g at the beginning of the experiment were housed singly in cages placed on three shelves within an experimental room on a normal L/D cycle (lights on: 08.00 h). Food was available between 13.00 h and 17.00 h and water was available ad lib. Wet mash (made by soaking 300 g chow with 450 ml water and blending to smooth consistency) was presented in clear 60 ml glass containers which were weighed before and after the meal. The meal was presented at either 10.00 or 11.00 h on a pseudo-random schedule to which the animals were accustomed for 7 days before the beginning of experimental observations.

During the final 2 days of the habituation period the animals were injected with saline at 09.30 h and their behaviour observed between 10.00 h and 10.50 h. The method was adapted from that described by Antin et al. (1975) and Kushner and Mook (1983). A microprocessor was programmed to illuminate a small LED next to one of the experimental cages; it was turned off by pressing one of four pushbuttons on a hand-held keyboard. Two and a half seconds later an LED next to the adjacent cage was illuminated and a key pressed; this process was repeated so that each of the 12 animals was observed every 30 s. Four mutually exclusive categories were used to record the behaviour; ingest; eat from the food dish or, very occasionally with a wet mash food, drink from the water bottle; groom; activities like grooming and licking that were directed at the body surface; explore; moving around the cage, rearing, sniffing or standing alert; rest; resting with head and body lowered, perhaps with the eyes closed. The observer recorded the behaviour occurring as the LED became illuminated, thus giving an unbiased estimate of the proportion of the time spent on the different activities (Altman 1974). The behavioural observations were summed into 5-min blocks for each animal and then subjected to an analysis of variance.

In experiment 3a 16 rats weighing between 241 g and 307 g at the beginning of the experiment were housed as in experiment 2 (lights on 07.00 h). They were habituated for 5 days to a deprivation cycle in which food was withdrawn at 09.00 h. Immediately after daily weighing at 15.30 h a meal of wet mash was presented for 30 min. On the final 2 habituation days all animals were injected with saline 30 min before the wet mash meal. Over a further 5-day period the animals were injected with either fluoxetine or saline; the two groups were matched for prior consumption of wet mash.

In experiment 3b 12 rats weighing between 210 and 285 g at the beginning of the experiment were habituated for 5 days to a deprivation cycle in which a wet mash meal was presented at 10.00 h and removed at 10.50 h; lights on was at 08.00 h. They were given weighed chow at 11.00 h and any not eaten was removed and weighed at 17.00 h. The animals were divided into two groups; the first was injected with fluoxetine 30 min before the wet mash meal on day 1 and with saline before the meals on the subsequent 2 days and the second group was injected with saline before the meal on each day. Group treatment was then reversed for a second 3-day cycle. Behavioural observations were made during the test meals as in experiment 2.

Results

Experiment 1: free feeding patterns

Fluoxetine led to a substantial reduction in food intake which was similar on both treatment days (fluoxetine: 335 pellets; saline: 495 pellets). There was no difference in water consumption (fluoxetine: 327 drinks; saline: 336 drinks).