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Tolerance pattern of the anorexigenic action of amphetamine in rats

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Summary

1. When food intake in fasted male rats was recorded daily for two consecutive 2 h periods it revealed a characteristic pattern.

2. In the control rats the food intake was greater during the first than the second 2 h period. Following (±)-amphetamine 5 mg/kg s.c. 30 min before food for 7 days there was a reversal of this normal food pattern. The rats consumed much less during the first 2 h period but progressively more during the second 2 h period.

3. The first 2 h food intake remained low throughout the treatment period and there was no evidence of development of tolerance despite continuation of treatment.

4. The second 2 h food intake steadily increased, contributing to the appearance of tolerance when only total 4 h food intake was recorded.

5. On withdrawal of amphetamine, there was immediate recovery of the first 2 h food intake. The second 2 h food intake, though decreased, remained high compared to the pretreatment level and may be responsible for the production of the 'rebound' phenomenon following withdrawal of amphetamine.

Introduction

An anorexigenic effect of amphetamine was observed as early as 1937, and a number of workers have confirmed this both in laboratory animals and in man (Ehrich & Krumbhaar, 1937; Nathanson, 1939; Tainter, 1944; Harris, Ivy & Searle, 1947). A rapid development of tolerance to the anorexigenic effect of amphetamine has also been reported in laboratory animals and in man (Tormey & Lasagna, 1960; Lawlor, Trivedi & Yelnosky, 1969; Goodman & Gilman, 1970). While carrying out studies on the appetite stimulating and depressing properties of drugs in rats, we noted a marked time dependence of the occurrence of tolerance following amphetamine; this is the subject of the present communication.

Methods

Male rats (mean weight range 170–180 g) were housed individually in metabolism cages and were observed for a total period of 3 weeks under similar environmental conditions. They were fasted throughout (water allowed *ad libitum*) except for a period of 4 h each day at a fixed time when food pellets (Hindlever) were offered. The food consumed during the first and second 2 h periods was measured

by weighing in a sensitive balance. In the first (pretreatment) week the animals were stabilized with regard to their food intake. At the beginning of the second (treatment) week the rats were divided randomly into two groups; one group consisting of 5 rats received amphetamine 5 mg/kg s.c., while the other group consisting of 6 rats received 0·9% w/v NaCl solution (saline), both 30 min before food for 7 days. The treatment was then stopped and the rats were observed for a further period of one (post-treatment) week.

The drug used was (±)-amphetamine sulphate (E. Merck) dissolved in normal saline and the dose is given in terms of the salt.

The statistical method employed in the analysis of results was the Student's *t* test.

Results

The mean 4 h food intake has been plotted daily for 3 weeks both for control and treated rats (Figure 1). The food intake stabilized by the third or fourth day of the pretreatment week in both the groups. There was a slight progressive

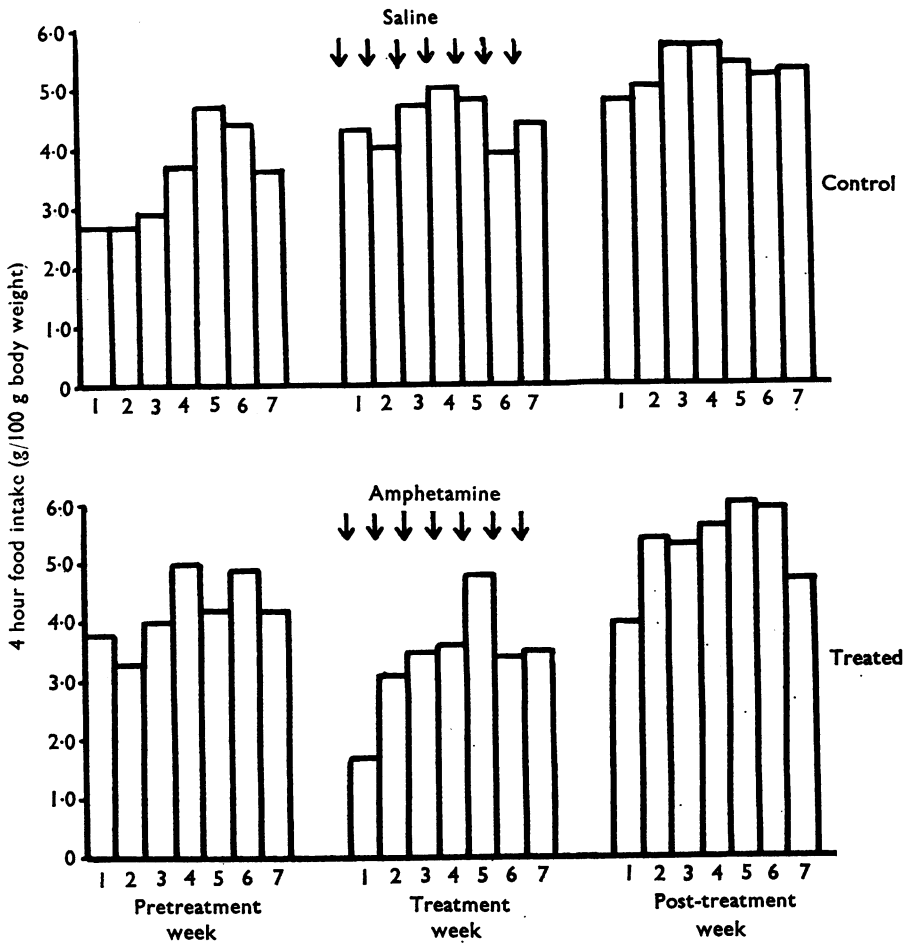

FIG. 1. Effect of (±)-amphetamine on 4 h food intake in fasted male rats. Ordinates: days in each week. At each arrow either (±)-amphetamine sulphate 5 mg/kg or saline was injected subcutaneously 30 min before food. Each column represents the mean of observations taken from 6 rats in the saline group and from 5 rats in the amphetamine group.

increase in the food intake during treatment and post-treatment weeks in the control series. However, in the treated series following amphetamine there was a marked decrease in the food intake on the first day. The effect was much less on the second day and by the fifth day the food intake was almost as much as in the pretreatment week, although subsequently there was another slight fall. On withdrawal of the drug there was not only complete recovery of food intake but some increase which persisted through most of the post-treatment week.

The mean first and second 2 h food intake values have also been plotted for both control and treated rats throughout the 3 week periods (Figure 2). The control rats during the whole experimental period and the treated rats during the pretreatment and post-treatment weeks consumed more food during the first 2 h

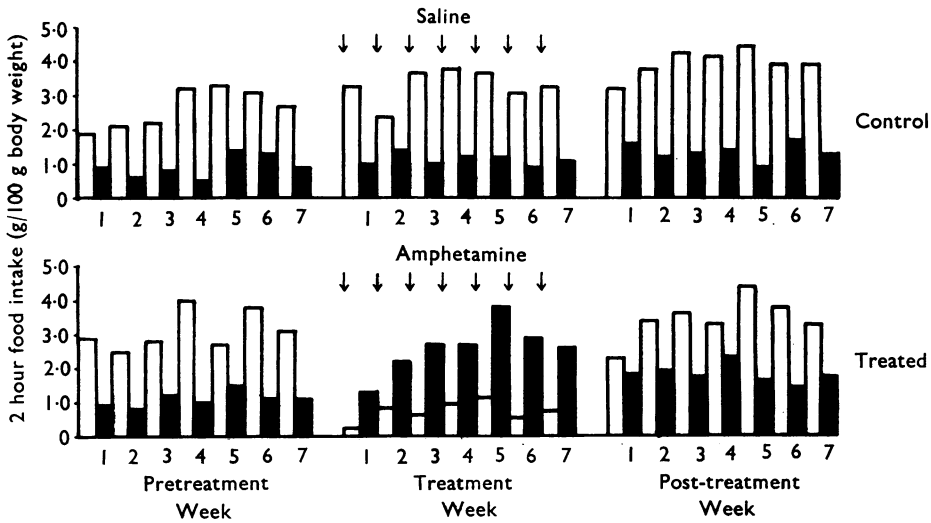

FIG. 2. Effect of (±)-amphetamine on 2 h food intake in fasted male rats. Open columns: first 2 h period; filled columns: second 2 h period. For further description, see Figure 1.

period than during the second. Following amphetamine, however, the pattern of food intake was completely reversed. In other words, the rats consumed much less food during the first 2 h period but there was an increase in the second 2 h food intake which was stabilized by the third day of treatment. This became more apparent when the results for the pretreatment and treatment weeks were pooled and differences between the means calculated (Table 1). As can be seen from the Table, the control rats ate slightly (but not significantly) more in both first and second 2 h periods in the treatment week than in the pretreatment week. Following amphetamine, the first 2 h food intake was significantly decreased ($P<0{\cdot}001$), while the second 2 h food intake increased ($P<0{\cdot}05$). It is also interesting to note that the first 2 h food intake remained at its lowest steady level right up to the end of the treatment week without any evidence of development of tolerance (Figure 2). Following withdrawal of amphetamine there was immediate recovery of the first 2 h food intake. It reached the pretreatment level by the second day bringing back once again the normal food pattern, that is, a greater food intake in the first 2 h than in the second. The second 2 h food intake, though decreased compared to the treatment week, remained higher than before treatment.

TABLE 1. *Effect of amphetamine on food intake during two consecutive 2 h periods in fasted rats*

Food intake g/100 g body weight (mean±S.E.M.)

	First 2 h			Second 2 h		
Treatment (No. of rats)	Pre-treatment week	Treatment week	Difference	Pre-treatment week	Treatment week	Difference
Control (6)	2·62±0·37	3·33±0·41	+0·71 N.S.	0·90±0·16	1·13±0·14	+0·23 N.S.
Amphetamine (5)	3·11±0·38	0·66±0·18	−2·45 $P<0·001$	1·10±0·43	2·64±0·40	+1·54 $P<0·05$

The treated group was injected daily with (±)-amphetamine sulphate 5 mg/kg s.c. 30 min before food for 7 days during the treatment week. The control group was injected with saline during the same period.

Discussion

The separate measurement of the first and second 2 h food intake in rats revealed an interesting property of amphetamine. Besides the well known reduction in food intake, and the tolerance to this, there was a change in the pattern of food intake. The food consumption in control rats was greater during the first 2 h period than the second 2 h period, while the reverse was true in amphetamine-treated rats. Another interesting point was that no tolerance developed during the first 2 h food intake, which remained depressed to the end of the week despite continued treatment with amphetamine. Our studies further revealed a steady increase in the second 2 h food intake which contributed to the apparent tolerance in total 4 h intake. Whether the increase in the second 2 h food intake was a compensatory mechanism secondary to the depressed intake during the first 2 h period, or a result of a direct appetite stimulating effect of amphetamine, is difficult to ascertain without further evidence. That it is not completely dependent on the first 2 h intake is apparent: although the first 2 h food intake increased markedly during the first two days after withdrawal of amphetamine, there was no proportionate decrease in the second 2 h intake. In fact, on the first day of the post-treatment week there was an increase in both the first and second 2 h food intake. We have also observed a 'rebound' phenomenon after treatment with the drug has ceased as reported by Shapiro & Freedman (1957) and by Tormey & Lasagna (1960). From our results it is evident that an increase in the second 2 h food intake has contributed substantially to this 'rebound' phenomenon.

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