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Paroxetine, a Selective 5-Hydroxytryptamine Uptake Inhibitor with Antidepressant Properties, Lacks Amphetamine-like Stimulus Properties in an Operant Drug Discrimination Bioassay in Rodents*

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Abstract—To evaluate whether the novel antidepressant paroxetine has any possible amphetamine-like actions, rats were trained to discriminate (+)-amphetamine sulphate in a standard two lever operant drug discrimination (DD) procedure using a fixed ratio 10 schedule of food reinforcement with a quantal, lever selection, index of the amphetamine stimulus. The 'training' dose of amphetamine was 1 mg kg^{-1}, i.p. Rats trained with this dose of amphetamine (n = 15) learned the drug discrimination rapidly over 30 training sessions and discriminative performance in these animals was subsequently maintained at a high level of accuracy (90% correct) over a prolonged time. In tests in these trained animals, amphetamine itself and the antidepressant agents nomifensine and tranylcypromine all produced clear, unequivocal dose-related generalization to amphetamine with ED50s of 0·2, 0·5 and 1·6 mg kg^{-1} respectively (as determined by probit analyses). In tests with paroxetine hydrochloride it was established that, over the dose range 0·3 to 10 mg kg^{-1}, no evidence was seen of generalization to the amphetamine stimulus. These data confirm earlier studies which suggested that some antidepressants may possess abuse potential because of their ability to induce amphetamine-like internal states. In contrast, paroxetine is devoid of such properties.

Antidepressant agents of various different types such as bupropion, nomifensine, selegiline and tranylcypromine can be associated with behavioural stimulant properties in animals (e.g. Costall et al 1975; Gerhards et al 1974; Jones et al 1980; Knoll & Magyar 1972; Kulkarni & Dandiya 1973). Some of these agents, such as tranylcypromine and nomifensine, have been reported to be abused by humans (Griffin et al 1981; Boning & Fuchs 1986) and to maintain drug self-administration in laboratory animals (Spyraki & Fibiger 1981). Therefore it now seems necessary to assess the abuse potential of novel antidepressants.

One assay procedure which has been used increasingly in recent years in the assessment of abuse liability of novel drugs is the drug discrimination (DD) procedure (Colpaert & Slangen 1982; Brady & Lukas 1984). In this behavioural assay, deprived animals are typically trained to respond on one of two levers to obtain food reward after drug injection, but to respond on the alternative lever when treated with the injection vehicle. The animal therefore has to discriminate its own internal state (i.e. to detect the drug 'cue') to decide which lever to select on any particular day. Typically, DD procedures provide highly specific bioassays in which animals discriminate specific cues causing them to generalize (i.e. to select the drug as opposed to the vehicle lever) *only* when treated with drugs with pharmacological properties in common with the so-called 'training' drug (see Colpaert & Slangen 1982). It has often been suggested (e.g. Colpaert 1986) that DD assays provide in-vivo measures of 'subjective' effects of drugs in animals and it is widely believed that such 'subjective' cueing effects of drugs may be good predictors of the abuse potential of specific agents (see e.g. Brady & Lukas 1984).

As far as studies with antidepressants are concerned, Colpaert et al (1980) reported that animals trained to discriminate cocaine generalized to a number of MAO inhibitors, although generalization was seen most readily with inhibitors of MAO type B. Similarly, Goudie (1982) reported that in animals trained to discriminate β-phenylethylamine, those animals which generalized consistently to amphetamine and cocaine also generalized to the MAO B inhibitor, selegiline ((−)-deprenyl). Porsolt et al (1982) reported that animals trained to discriminate amphetamine, which has similar properties in DD assays to cocaine (Stolerman & D'Mello 1981), also showed generalization to a number of MAO inhibitors, and to atypical antidepressants such as bupropion and nomifensine. In a subsequent study, Porsolt et al (1984) confirmed that a number of MAO inhibitors generalized to amphetamine, although by no means all such drugs generalized, even some potent inhibitors of MAO type B failed to show up as amphetamine-like in these studies. Jones et al (1980) reported that animals trained to discriminate the atypical antidepressant bupropion generalized to amphetamine, cocaine and other CNS stimulants. These animals also generalized to the atypical antidepressants viloxazine and nomifensine.

Collectively, these data show that some, but by no means *all*, clinically effective antidepressants of different types, act like CNS stimulants in DD assays. In conjunction with the reported self-administration of some antidepressants by animals (Spyraki & Fibiger 1981) and the reported abuse of some such agents in humans (Griffin et al 1981; Boning & Fuchs 1986), the data reviewed above emphasise the need to screen potential novel antidepressants for possible amphetamine-like properties that might lead to abuse. The present

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study was concerned with such an analysis of the actions of paroxetine, a potent and selective 5-hydroxytryptamine (5-HT) uptake inhibitor (Buss Lassen 1978a). Paroxetine is a clinically effective antidepressant (Lund Laursen et al 1985) which does not induce amphetamine-type stereotypy (A. M. Johnson, personal communication). It does however produce weak stimulation of locomotor activity (Johnson et al 1985), although only at doses substantially greater than those required to inhibit 5-HT uptake in-vivo (Buss Lassen 1978a, b). Thus, although paroxetine does not appear to activate dopaminergic systems in-vivo (Johnson et al 1985), because of the reported abuse potential of a number of different types of antidepressants and because of the weak stimulant actions of paroxetine, it was considered necessary to determine whether paroxetine possesses amphetamine-like actions in animals trained to discriminate amphetamine. This assessment of paroxetine was also thought necessary in the light of evidence (Saletu et al 1986) that some selective 5-HT reuptake inhibitors can improve psychomotor performance and increase critical flicker fusion frequency in humans. Such evidence of CNS activation *might* be associated with amphetamine-like abuse.

Methods

Animals

Fifteen female hooded rats (220–260 g), derived from the breeding stock of Liverpool University Psychology Department, were individually housed in a temperature (21°C)-controlled room. Each subject was maintained at about 80% of its free feeding level by daily supplementary feeding. Subjects were run in operant sessions on 5 to 7 days each week.

Apparatus

Standard operant chambers (Colbourn Instruments, USA), containing two levers, were used. A food chamber was located between the levers and reinforcement consisted of 45 mg pellets of food. A light in the food dipper came on for 160 ms during the presentation of each pellet, providing secondary reinforcement. Presentation of light stimuli and pellets and recording of behavioural responses was achieved with a minicomputer.

Training procedure for acquisition of amphetamine/saline discrimination

The procedure was similar to the fixed ratio (FR10) drug discrimination procedure described by Goudie et al (1986). Subjects were initially trained to press either lever for food reward. Subsequent operant sessions were of 15 min duration and only one lever was operative on any one day. The single operative lever was determined by whether the subjects were injected with amphetamine or saline. Injections were administered before each session in the two-weekly random sequence described by Goudie et al (1986). The dose of (+)-amphetamine sulphate administered during discrimination training was 1 mg kg^{-1} and was selected on the basis of previous studies which have shown that it produces a centrally mediated cue which is specific for CNS stimulants (Stolerman & D'Mello 1981; Goudie et al 1986). The sequence of operative levers was randomized between successive subjects in each operant chamber to avoid the procedure being confounded by olfactory cues between animals (Extance & Goudie 1981). For half the subjects the drug lever was the left lever and for half the subjects it was the right lever. The schedule of reinforcement on the operative lever was progressively escalated from fixed ratio 1 (FR1) to fixed ratio 10 (FR10), injections of drug or saline preceding each session. Subsequently, the fixed ratio 10 (FR10) schedule was used for the remainder of the study. On all training and test sessions the total number of responses on both levers was recorded. Accuracy of lever selection on each session was assessed in terms of the total number of responses accumulated on both levers prior to the delivery of the first reward; this was defined as the FRF value (cf. Colpaert et al 1976). All subjects were trained to a criterion of *at least* 8 out of 10 consecutive sessions of correct lever selection (FRF $\leqslant 19$). All subjects were then carried forward after 30 training sessions to the test phase of the study in which generalization and substitution tests were conducted.

Test phase

Test sessions were always run with at least one (and usually more) intervening baseline training sessions. On test days the subjects were only reinforced throughout the operant session for responding on the lever on which they first accumulated 10 responses—the 'selected lever'. On intervening days the baseline training sessions were continued to maintain accurate discriminative control by the training drug. This procedure was effective in ensuring that lever selection never fell below the 90% correct level. Immediately before some planned test sessions, some subjects occasionally made errors in lever selection, these being defined as occurring if the FRF value was > 19. If a subject made an error, that subject was not tested on the subsequent planned test day. Some animals were discarded from the study towards its completion as they developed respiratory infections. Thus, test data were sometimes reported on the basis of all 15 trained subjects and sometimes the data refer to only 10 trained subjects. No test involved less than 10 subjects. Initial tests were concerned with establishing a dose-effect curve for the amphetamine cue. Subjects were injected with various doses of amphetamine prior to test sessions. Subsequently, the ability of a number of doses of various drugs to substitute for the amphetamine cue were tested.

Drugs

(+)-Amphetamine sulphate (Smith, Kline and French), nomifensine maleate (Hoechst) and tranylcypromine hydrochloride (Sigma) were dissolved in isotonic saline. Paroxetine hydrochloride (Beecham Pharmaceuticals) was suspended in 1% methylcellulose in saline. All drug treatments were given at an injection volume of 1 mL kg^{-1}, 30 min before operant sessions, all injections being i.p. Doses of amphetamine, nomifensine and tranylcypromine were expressed as the salt, paroxetine as the base. During tests with any one drug the doses that were administered were given in random order. Drugs were tested in a non-systematic order over a period of about 4 months after discrimination training.

Data analyses

Lever-selection data obtained with drugs that generalized to

the amphetamine cue were analysed using probit analysis techniques based on maximum likelihood estimates (Finney 1952). Effects of drugs on operant response rates were analysed by expressing response rates under drugs as percentages of response rates obtained during the most immediately preceding session with saline—the 'response level' (cf. Colpaert et al 1976). These response level data were analysed by repeated measures ANOVAs followed by Tukey HSD multiple comparison tests.

Results

The data relevant to the acquisition of the amphetamine discrimination over the first 30 training trials are shown in Fig. 1. Data from amphetamine and saline training sessions are plotted separately. Initially, animals responded randomly (i.e. lever selection was at the 50% chance level). However, it is clear that amphetamine rapidly acquired a high level of stimulus control over lever selection, so that after 30 training trials lever selection was virtually perfect after injection of both amphetamine and saline. By trial 30, all 15 animals had learned the discrimination to a criterion of *at least* 8 correct lever selections out of 10 consecutive sessions. (Although a large number of animals had been trained to much more stringent criteria. Thus by trial 30, 9 of the 15 animals (60%) had reached a criterion of 14 out of 15 consecutive correct lever selections.)

The results of the amphetamine, nomifensine and tranylcypromine generalization tests are shown in Fig. 2. At the lowest dose of amphetamine tested, only one of 15 trained rats selected the drug lever. With progressively increasing doses of amphetamine, dose-related selection of the drug lever was seen. At the training dose (1 mg kg^{-1}) and half the training dose (0·5 mg kg^{-1}) all 15 trained rats selected the drug lever, demonstrating the very reliable control over lever selection exerted by amphetamine in these trained rats. Probit analysis (see Fig. 2) produced an estimated ED50 for amphetamine generalization of 0·21 mg kg^{-1} (0·16 to 0·27, 95% confidence limits). The probit line provided a good fit to these data ($Chi^2 = 5·14$, $P > 0·05$).

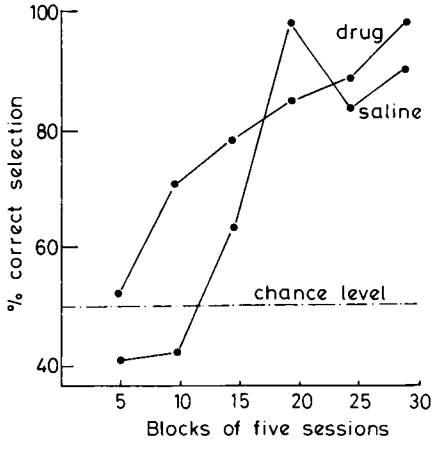

FIG. 1. Acquisition of the amphetamine/saline discrimination. The data shown are the percentage of correct lever selections in saline and amphetamine (drug) training sessions for the first 30 training sessions, plotted in blocks of five sessions with 15 animals being trained. Note how lever selection was initially at chance levels (50% correct) but that the proportion of correct lever selections increased progressively with training trials.

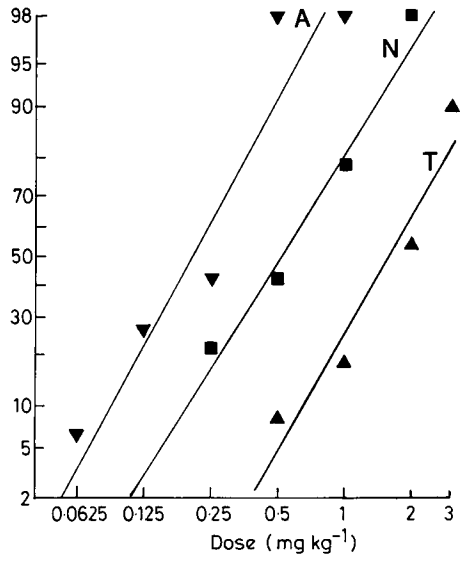

FIG. 2. Log/probit plots for the amphetamine (A), nomifensine (N) and tranylcypromine (T) generalization data. The theoretical regression lines are plotted as are the raw data points for each drug. Note that as it is not possible to plot 100% on a probit scale, such data points are arbitrarily plotted as 98% drug lever selection.

The results of the generalization tests conducted with nomifensine are also plotted in Fig. 2. The ED50 for generalization was 0·51 mg kg^{-1} (0·35–0·69, 95% confidence limits). The plotted probit line provided a good fit for the data ($Chi^2 = 1·01$, $P > 0·05$). The data show unequivocally that nomifensine produced dose-related generalization to the amphetamine cue and that the observed generalization resulted in parallel dose/response curves. The results of the generalization tests conducted with tranylcypromine indicated that the ED50 for generalization was 1·61 mg kg^{-1} (1·16 to 2·37 95% confidence limits). The plotted probit line again provided a good fit for the data ($Chi^2 = 1·40$, $P > 0·05$). These data again show unequivocally that tranylcypromine produced dose-related generalization to the amphetamine cue and that the observed generalization resulted in parallel dose/response curves (see Fig. 2).

In a paroxetine vehicle control test (with methylcellulose in saline) all 15 rats tested selected the saline lever ($P < 0·001$, binomial test), providing further evidence of the high degree of accuracy of lever selection in this study. In 4 different paroxetine generalization tests (with doses of 0·3, 1·0, 3·0 and 10·0 mg kg^{-1}), each of which was conducted with at least 14 trained rats, there was minimal evidence of generalization to amphetamine. The *only* case of drug lever selection observed in these paroxetine tests was seen at 10 mg kg^{-1} in one rat out of 14 tested. These data show convincingly that, in a relatively large sample of subjects, paroxetine did not generalize to the amphetamine cue over a wide range of doses.

The effects of amphetamine and other drugs on response rates in the generalization tests are shown in Table 1. Statistical analysis of the data obtained in the five amphetamine tests (Table 1) showed that there was a significant effect

Table 1. Response rate data obtained in generalization tests with various drugs.

Drug	Dose (mg kg^{-1})	Response rate (% of saline baseline) Mean	s.e.
Amphetamine	0·0625	78·7	5·0
	0·125	113·7	10·1
	0·25	95·5	5·5
	0·5	118·7	15·0
	1·0	123·9	12·3
Paroxetine	0 (vehicle control)	90·6	2·4
	0·3	75·4	1·6
	1·0	87·1	3·3
	3·0	73·9	4·1
	10·0	75·4	5·2
Nomifensine	0·25	106·3	12·6
	0·5	98·7	10·6
	1·0	112·8	5·6
	2·0	117·0	6·1
Tranylcypromine	0·5	90·5	7·8
	1·0	86·7	6·1
	2·0	48·6	9·0
	3·0	61·1	7·4

of amphetamine dose on response level ($F = 3{\cdot}45$, df = 4, 56, $P < 0{\cdot}025$). Subsequent multiple comparison tests revealed that the response level under 0·0625 mg kg^{-1} amphetamine differed significantly ($\alpha = 0{\cdot}05$) from that under 0·5 and 1 mg kg^{-1}, no other comparisons being significant. Thus amphetamine produced a dose-related increase in response rate relative to that seen at the lowest dose tested, this increase was most pronounced at higher doses.

Table 1 shows that paroxetine appeared to suppress operant responding. Analysis of the paroxetine test data in conjunction with the paroxetine vehicle control data revealed a significant effect of dose on response level ($F = 4{\cdot}01$, df = 4, 52, $P < 0{\cdot}01$). Subsequent multiple comparison tests ($\alpha = 0{\cdot}05$) revealed, however, that the only significant pairwise comparisons were between the vehicle control data and the effects of 0·1, 1 and 10 mg kg^{-1} of paroxetine. No comparisons between different doses of paroxetine were significant. Thus paroxetine suppressed response rate, although the effect was *not* clearly dose related. It is clear, however, that the effect of paroxetine on response level differed from that of amphetamine.

The effects of nomifensine on response rate revealed that nomifensine generalized to amphetamine at doses which did not suppress responding. Statistical analysis of the data obtained in the four nomifensine tests (Table 1) showed that there was no significant effect of nomifensine dose on response level (repeated measures ANOVA. $F < 1$, df = 3, 36).

The effects of tranylcypromine on response rate demonstrated that tranylcypromine only showed high levels of generalization to amphetamine at doses which had relatively marked suppressant effects on response rate. In this respect, tranylcypromine differed from nomifensine. Statistical analysis of the data obtained in the four tranylcypromine tests (Table 1) showed that there was a highly significant effect of dose on response level (repeated measures ANOVA. $F = 17{\cdot}22$, df = 3, 27).

Discussion

The data reported show clearly that paroxetine does not possess amphetamine-like stimulus properties, although such properties were found in the MAO inhibitor tranylcypromine and in the antidepressant nomifensine. In other studies (data not shown) we have confirmed the finding that paroxetine at doses of 1 and 3 mg kg^{-1} does not generalize to amphetamine and we have also shown that such doses of paroxetine do not shift the amphetamine generalization curve either to the left or to the right. Thus it appears that paroxetine fails to generalize to amphetamine or to modify the amphetamine stimulus even at doses substantially above those which have been reported to inhibit 5-HT uptake in-vivo (Buss Lassen 1978 a,b). Thus it is clear that at doses at which the drug is likely to be used as an antidepressant, abuse of the amphetamine type is almost certain to be absent. As noted by Porsolt et al (1984), although the possession of amphetamine-like stimulus properties is not an unequivocal indication that a specific drug will be abused in man, the absence of such properties is clearly an advantage in an antidepressant.

The potency of drugs in generalizing to amphetamine was not correlated with their actions on operant responding, since nomifensine generalized to amphetamine at doses which did not suppress responding whilst tranylcypromine only generalized at doses which suppressed responding substantially. Thus it is clear that in this study animals were not simply detecting non-specific motoric actions of drugs on rates of responding, instead it would appear that animals were detecting specific internal amphetamine-like stimuli.

It is also clear from the data reported above that amphetamine and paroxetine differed in their actions on rates of operant responding, since paroxetine suppressed responding whilst amphetamine enhanced it. These observations provide a further differentiation between the actions of amphetamine and paroxetine.

The finding that nomifensine and tranylcypromine generalized to the amphetamine stimulus is compatible with the data reviewed above which indicates that a number of antidepressants may be prone to abuse due to their amphetamine-like actions (Colpaert et al 1980; Jones et al 1980; Porsolt et al 1982, 1984). The generalization to amphetamine seen with tranylcypromine may well be related to the close structural similarity between this agent and amphetamine, it being known that tranylcypromine will cause locomotor stimulation of the amphetamine type (Smith 1980). The generalization seen with nomifensine seems most likely to be explicable in terms of the known actions of this drug on brain dopaminergic systems (Costall et al 1975), since there is evidence that the amphetamine stimulus is mediated, at least in part, by the dopaminergic system (Colpaert et al 1978).

In summary, the data show that, over the dose range tested, paroxetine is devoid of amphetamine-like stimulus properties. In contrast, nomifensine and tranylcypromine produced dose-related generalization to the amphetamine cue. It is clear that drug discrimination procedures can be used to detect amphetamine-like cue properties of potential antidepressants (cf. Porsolt et al 1982, 1984) and that paroxetine does not possess such properties.

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References

Boning, J., Fuchs, G. (1986) Pharmacopsychiatry 19: 386–388

Brady, J. V., Lukas, S. E. (1984) NIDA Research Monograph 52. U.S. Government Printing Office, Washington

Buss Lassen, J. (1978a) Psychopharmacology 57: 151–153

Buss Lassen, J. (1978b) Eur. J. Pharmacol. 47: 351–358

Colpaert, F. C. (1986) in: Goldberg, S. R., Stolerman, I. P. (eds) Behavioral Analysis of Drug Dependence. Academic Press, New York, pp 161–193

Colpaert, F. C., Slangen, J. L. (eds) (1982) Drug Discrimination: Applications in CNS Pharmacology. Elsevier Biomedical Press, Amsterdam

Colpaert, F. C., Niemegeers, C. J. E., Janssen, P. A. J. (1976) Psychopharmacology 46: 169–177

Colpaert, F. C., Niemegeers, C. J. E., Janssen, P. A. J. (1978) Neuropharmacology 17: 937–942

Colpaert, F. C., Niemegeers, C. J. E., Janssen, P. A. J. (1980) Pharmacol. Biochem. Behav. 13: 513–517

Costall, B., Kelly, D. M., Naylor, R. J. (1975) Psychopharmacology 41: 153–164

Extance, K., Goudie, A. J. (1981). Ibid. 73: 363–371

Finney, D. J. (1952) Probit Analysis. Cambridge University Press, London

Gerhards, H. J., Carenzi, A., Costa, E. (1974) Naunyn Schmeideberg's Arch. Pharmacol. 286: 49–63

Goudie, A. J. (1982) in: Colpaert, F. C., Slangen, J. L. (eds) Drug Discrimination: Applications in CNS Pharmacology. Elsevier Biomedical Press, Amsterdam, pp 165–180

Goudie, A. J., Atkinson, J., West, C. R. (1986). Neuropharmacology 25: 85–94

Griffin, N., Draper, R. J., Webb, M. G. T. (1981) Br. Med. J. 283: 346

Johnson, A. M., Nelson, D. R., Thomas, D. R. (1985). Poster presented to 4th World Congress of Biological Psychiatry. Philadelphia, USA.

Jones, C. N., Howard, J. L., McBennett, T. (1980) Psychopharmacology 67: 111–118

Knoll, J., Magyar, K. (1972) in: Costa, E., Sandler, M. (eds) Monoamine Oxidases-New vistas. Raven Press, New York: pp 393–408

Kulkarni, S. K., Dandiya, P. C. (1973) Psychopharmacology 33: 333–338

Lund Laursen, A., Mikkelsen, P. L., Rassmussen, S., Le Fevre Honore, P. (1985) Acta Psychiat. Scand. 71: 249–255

Porsolt, R. D., Pawelec, C., Jalfre, M. (1982) in: Colpaert, F. C., Slangen, J. L. (eds) Drug Discrimination: Applications in CNS Pharmacology. Elsevier Biomedical, Amsterdam, pp 193–202

Porsolt, R. D., Pawelec, C., Roux, S., Jalfre, M. (1984) Neuropharmacology 23: 569–573

Saletu, B., Grunberger, J., Linzmayer, L. (1986) J. Neural Trans. 67: 241–266

Smith, D. F. (1980) Pharmakopsychiatrie 13: 130–136

Spyraki, C., Fibiger, H. C. (1981) Science 212: 1167–1168

Stolerman, I. P., D'Mello, G. D. (1981). Psychopharmacology 73: 295–303