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Effects of Norepinephrine and Serotonin Uptake Inhibitors on the Schedule-Controlled Behavior of the Pigeon

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LEANDER, J. D. AND R. B. CARTER. *Effects of norepinephrine and serotonin uptake inhibitors on the schedule-controlled behavior of the pigeon.* PHARMACOL BIOCHEM BEHAV **20**(3) 391–395, 1984.—The effects of nortriptyline, amitriptyline, desipramine, chlorimipramine, protriptyline, doxepin, nisoxetine, fluoxetine and iprindole were determined on responding by pigeons under a multiple fixed-ratio 30-response, fixed-interval 10-minute schedule of grain presentation. Those drugs which have been shown to block uptake of norepinephrine decreased fixed-interval quarter-life values. Those which are considered most selective as norepinephrine uptake inhibitors also increased overall fixed-interval responding. These increases in fixed-interval responding, both on local and overall rates, in pigeons appear to be due to the actions of these drugs to inhibit uptake of norepinephrine rather than to other actions.

Antidepressants Norepinephrine uptake inhibitors Multiple fixed ratio, fixed-interval Operant behavior Pigeons

THE tricyclic antidepressant imipramine produces marked increases in responding under fixed-interval schedules of food presentation in pigeons [2, 15, 20–22]. Recently, McKearney [16] reported that several of the tricyclic antidepressants increased responding in some individual squirrel monkeys, and that the relative potencies to increase responding corresponded closely to the anticholinergic potencies of these tricyclic antidepressants (imipramine, amitriptyline, nortriptyline, chlorimipramine and desipramine). This point was made by demonstrating that the same individual monkeys that exhibited rate-increasing effects with the tricyclics also exhibited rate-increasing effects with common anticholinergic drugs, such as scopolamine and atropine [16]. In the pigeon, however, anticholinergic drugs (benztropine, scopolamine and atropine) do not increase responding under fixed-interval schedules [14]. Thus it is unlikely that the anticholinergic properties of the tricyclics are responsible for the rate-increasing effects in pigeons.

The purposes of the present experiment were to determine the generality of the rate-increasing effects of imipramine in the pigeon by studying the effects of a variety of the tricyclic antidepressants, desipramine, chlorimipramine, protriptyline, doxepin, nortriptyline and amitriptyline. Since the N-dimethyl-substituted tricyclic compounds (amitriptyline, doxepin and chlorimipramine) are rapidly metabolized *in vivo* to the monodesmethyl compounds (nortriptyline, desmethyldoxepin and chlordesmethylimipramine) [10,26] with different uptake-inhibiting properties, each N-dimethyl-substituted compound was compared to a monodesmethyl compound. Thus amitriptyline was compared to nortriptyline; doxepin was compared to protriptyline, and chlorimipramine was compared to desipramine. In general, the monodesmethyl compounds preferentially block uptake of norepinephrine compared to serotonin, whereas the reverse is true for the dimethyl compounds [11, 18, 19]. However, when a dimethyl-substituted compound is administered, the behavioral effects are due both to it and the monodesmethyl metabolite [10]. Fluoxetine, a specific serotonin uptake inhibitor [4,24], and nisoxetine, a specific norepinephrine uptake inhibitor [23,25] were also studied. Iprindole, a tricyclic antidepressant lacking inhibitory effects on uptake, was also included for study [5,11]. The present results show that drugs which block the uptake of norepinephrine increase the low rates of responding that occur at the beginning of fixed-interval compounds, and this can result in overall increases in fixed-interval rates.

METHOD

Male White Carneaux pigeons, maintained under standard conditions, responded under a multiple fixed-ratio 30-response, fixed-interval 10-minute (mult FR, FI) schedule of

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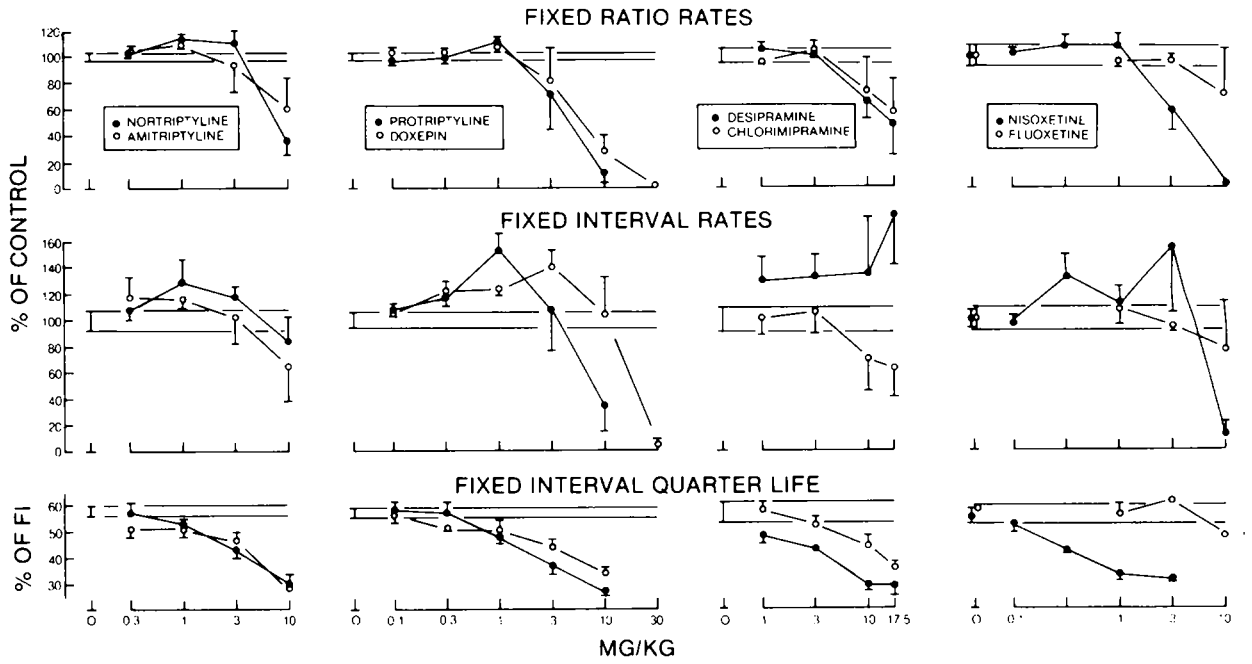

FIG. 1. Mean effects of the eight uptake inhibitors on the rates of responding in the FR component (top row) and in the FI component (middle row) and on the FI quater-life value (bottom row). Abscissae show dose of drug; ordinates show rates of responding in FR and FI as percent of control rate and show FI quarter-life value as percent of FI. The points and brackets show mean±S.E. for 4 birds, with each bird receiving each dose twice. Each comparison of two drugs (nortriptyline and amitriptyline; protriptyline and doxepin; desipramine and chlorimipramine) except fluoxetine and nisoxetine occurred in the same group of 4 birds each. Fluoxetine and nisoxetine were each studied in separate groups of 3 and 4 birds, respectively. The horizontal lines through each frame of the figure show the ±S.E. (n=number of birds, usually 4) for the distilled water control injection data.

grain presentation [12–14]. Two-hour sessions were conducted Monday through Friday, with drug injections administered usually on Tuesdays and Fridays. The drugs used and the forms in which the doses were calculated were nortriptyline HCl, amitriptyline HCl, protriptyline HCl, doxepin HCl, desipramine HCl, chlorimipramine HCl, nisoxetine HCl (LY94939), fluoxetine HCl (LY110140) and iprindole HCl. All compounds were dissolved in distilled water. All injections were administered in a volume of 1 ml/kg body weight into the breast muscle 10 min before the onset of the test sessions. Injections of distilled water were administered on Thursdays, and the data obtained on Thursdays were used as non-drug control data against which to evaluate the drug data. Average rates of responding in each component of the multiple schedule were calculated for each bird, and then drug effects were calculated as a percent of each bird's control values. The data are presented as the mean±standard error for 4 birds. The distribution of responses within the FI component of the multiple schedule was obtained by dividing the interval into ten 30-sec segments and recording the number of responses in each of the segments. These data were used to calculate the FI quarter-life value, a statistic which is independent of response rate and which is used to describe quantitatively the pattern of responding that occurs under the FI schedule. The quarter-life value is the proportion of the interval duration required for the bird to emit 25 percent of the total responses in the FI [6]. Quarter-life values around 50 percent of the FI indicate that the pattern of responding under the FI schedule was positively accelerated, with 75 percent of the total responding occurring in the last 50 percent of the interval. The data from the ten 30-sec segments were used to quantify the effects of the drugs on the local rates of responding within the FI. This was done by expressing the mean absolute rate of responding after drug as a function of the absolute rate of responding after the water injection [7].

RESULTS

Figure 1 shows the mean dose-effect data for the uptake inhibitors. In the far left column are the data obtained with nortriptyline and amitriptyline. Both produced negligible effects on FR rates except to decrease rates after the highest dose (10 mg/kg) of each drug. The FI rates show a similar pattern except that the decreases produced by the 10 mg/kg doses were less than in the FR. The most reliable effect of these two drugs was to markedly decrease the quarter-life value as a function of increasing dose.

Protriptyline and doxepin (second column from left) decreased FR rates of responding as a function of increasing dose (>3 mg/kg). In the FI component, both protriptyline and doxepin increased rates of responding at intermediate doses (at 1 and 3 mg/kg, respectively) and then decreased FI rates at higher doses. Protriptyline appeared clearly more potent than doxepin in decreasing FI responding, but they were approximately equally potent in decreasing FR re-

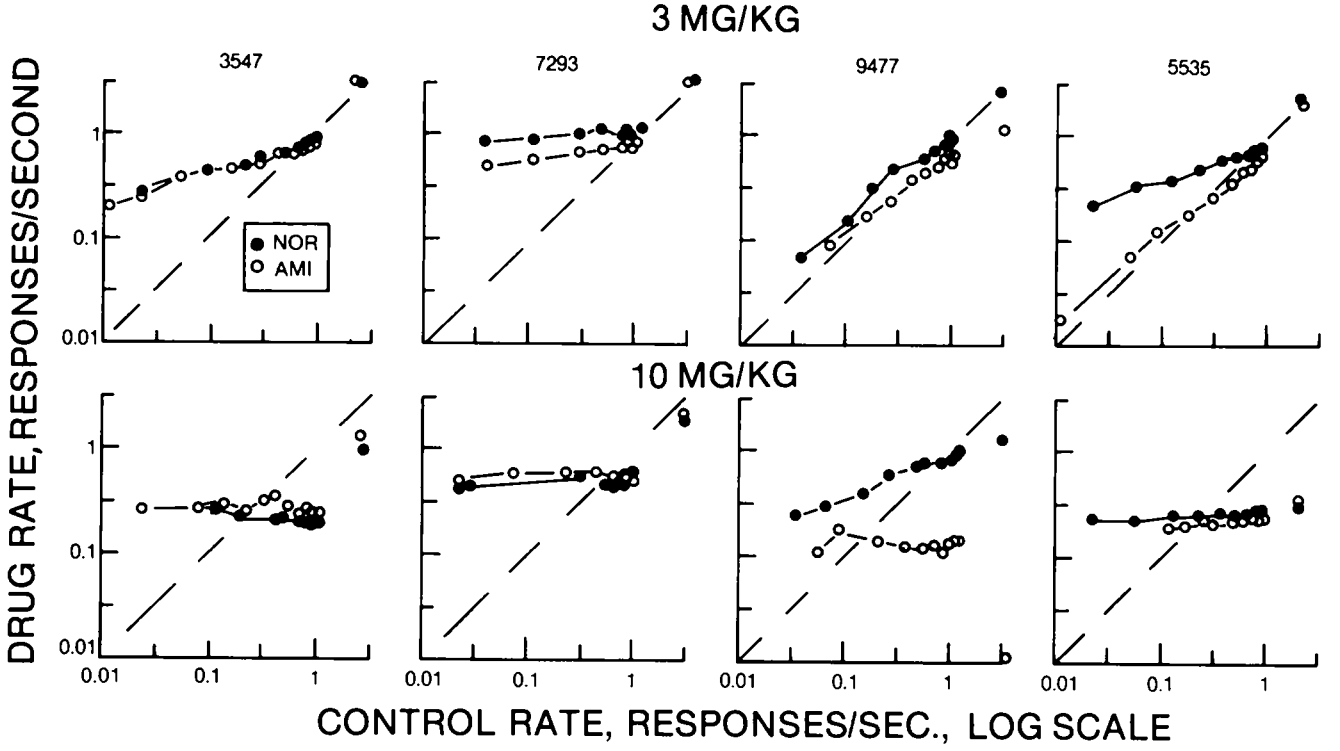

FIG. 2. Effects of 3 and 10 mg/kg of nortriptyline and amitriptyline on the local rates of responding within the FI and on the FR rate plotted as a function of the rates after the associated water injection for each of 4 individual pigeons (number 3547, 7293, 5477 and 5535). Abscissae show rates of responding after the water injection as responses per second. Ordinates show rates of responding after drug. Points above and to the left of the dashed diagonal line indicate increases, whereas points below and to the right indicate decreases in local rates of responding compared with non-drug control performance. The filled circles show the effects of nortriptyline, and the unfilled circles show the effects of amitriptyline. The points to the far right in each frame are the FR rates. No point was plotted if either the control or drug rate for the 30-sec segment was less than 0.01 response/sec.

sponding. Both protriptyline and doxepin decreased quarter-life value as a function of increasing dose.

As with the previous drugs compared, both desipramine and chlorimipramine (second column from the right) decreased FR rates of responding at the higher doses, and were approximately equally potent in producing this effect. However, in the FI component, desipramine and chlorimipramine produced qualitatively different effects: increases in responding after desipramine and decreases after the higher doses (10 and 17.5 mg/kg) of chlorimipramine. Like the other uptake inhibitors, both desipramine and chlorimipramine produced dose-related decreases in quarter-life value, with desipramine being about 3 times more potent than chlorimipramine.

The effects of nisoxetine and fluoxetine are shown on the far right in Fig. 1. Nisoxetine decreased FR responding after 3 and 10 mg/kg, whereas there was an increase in FI responding after 3 mg/kg, and a marked suppression of FI responding after 10 mg/kg. The large variability at 3 mg/kg was due to the fact that two birds had FI response rates of 275 and 205 percent of control, whereas the other two had response rates of 64 and 75 percent of control. Of the two birds that exhibited decreases in FI responding after 3 mg/kg, one had an FI rate of 182 percent after 0.3 mg/kg, whereas the second bird never had an increase in FI responding after nisoxetine. The lack of FI increase in this bird might be due to a relatively high control rate (0.84 responses/sec) in the FI component. There was also a dose-related decrease in FI quarter-life value with clear decreases with nisoxetine doses as low as 0.3 mg/kg. In contrast to the effects of nisoxetine, there were few effects of fluoxetine with doses as high as 10 mg/kg.

Iprindole was studied in two birds (data not shown) to determine if its effects would be similar to the uptake inhibitors. A dose of 100 mg/kg decreased responding in both schedule components in both birds, whereas doses of 30 mg/kg and less were without effect on any measure, including quarter-life value.

As a further analysis of the effect of these compounds on the patterning of responding during the FI, Fig. 2 shows the effects of two doses (3 mg/kg, top, and 10 mg/kg, bottom) of nortriptyline and amitriptyline in the four individual birds on the local rates of responding within the FI and on the FR rate plotted as a function of the non-drug control rate. After the 3 mg/kg dose of both nortriptyline and amitriptyline, there were increases in the lower rates of responding that typically occurred at the beginning of the FI, without reliable decreases in the higher rates from the terminal segments of the FI or the FR rates. After 10 mg/kg of both compounds, every animal exhibited essentially constant rates of responding throughout the FI. This constant rate of responding within

the FI is a product of increasing the low rates of responding from early in the interval and decreasing the high rates from the terminal parts of the FI, and results in the quarter-life value approaching 25 percent of the FI. Thus the dose-related decreases in FI quarter-life value for the drugs shown in Fig. 1 can be generalized as a tendency of those drugs to increase low rates and decrease high rates of responding to approach a constant rate of responding within the FI.

DISCUSSION

The present results show that all of the tricyclic uptake inhibitors decrease the positively accelerated patterning of responding under the FI schedule to produce marked dose-related decreases in the quarter-life values. Analysis of the local rates of responding indicated that these decreases in quarter-life values were a result of increases in the low rates of responding at the beginning of the FI and decreases in the high rates at the terminal segments of the FI. With some of these compounds, these increases in low rates of responding resulted in increases in the overall rates of FI responding. Thus these data extend the generality of the results reported earlier for imipramine in the pigeon [2, 15, 20–22] to other imipramine-like, tricyclic antidepressants. The present results also show that nisoxetine, a relatively selective norepinephrine uptake inhibitor [23,25], decreases quarter-life value and increases overall FI rates, whereas fluoxetine, a selective serotonin uptake inhibitor [4,24], does not affect the schedule-controlled behavior. Fluoxetine does block serotonin uptake at the doses tested in this experiment, as has been demonstrated by giving fluoxetine along with a serotonin precursor [1,9]. The similarities between nisoxetine and desipramine, on the one hand, and fluoxetine and chlorimipramine, on the other, support the hypothesis that the rate-increasing effects are due to norepinephrine uptake inhibition. Iprindole, an antidepressant which does not block uptake of either norepinephrine or serotonin, did not affect FI patterning or rates of responding until very high doses (100 mg/kg) were administered.

The order of potency for decreasing the quarter-life values (to 40 percent of FI and calculated from linear regression analysis) were as follows, from most potent to least: nisoxetine>protriptyline=desipramine>nortriptyline>amitriptyline>doxepin>chlorimipramine>>iprindole and fluoxetine. This potency order correlates well with the activity of these compounds to block *in vitro* uptake of norepinephrine in rat tissue [11] and not with their activity to block uptake of serotonin or to block cholinergic receptors [3, 8, 11]. The potency order for anticholinergic activity in human brain tissue is: amitriptyline=protriptyline>doxepin>nortriptyline=desipramine>>fluoxetine, iprindole, nisoxetine [3]. Thus, in the pigeon, it appears to be the activity to block uptake of norepinephrine which produces "stimulatory-like" effects on FI schedule performance, whereas in squirrel monkeys it may be the anticholinergic actions which increase FI responding [16]. Thus this is an interesting example where a class of drugs produces a similar pattern of effects across two different species but by different mechanisms. In contrast with the results in pigeons, norepinephrine uptake inhibitors do not increase low rates of responding in rats [15,17].

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REFERENCES

1. Carter, R. B. and J. D. Leander. Effect of serotonin uptake inhibition on the behavioral effects of 5-hydroxytryptophan (5-HTP) and quipazine. *Fed Proc* **39:** 608, 1980.
2. Dews, P. B. A behavioral output enhancing effect of imipramine in pigeons. *Int J Neuropharmacol* **1:** 265–272, 1962.
3. El-Fakahany, E. and E. Richelson. Antagonism by antidepressants of muscarinic acetylcholine receptors of human brain. *Br J Pharmacol* **78:** 97–102, 1983.
4. Fuller, R. W. and D. T. Wong. Inhibition of serotonin reuptake. *Fed Proc* **36:** 2154–2158, 1977.
5. Gluckman, M. I. and T. Baum. The pharmacology of iprindole, a new antidepressant. *Psychopharmacologia* **15:** 169–185, 1969.
6. Gollub, L. R. The relations among measures of performance on fixed-interval schedules. *J Exp Anal Behav* **7:** 337–343, 1964.
7. Gonzales, F. A. and L. D. Byrd. Mathematics underlying the rate-dependency hypothesis. *Science* **195:** 546–550, 1977.
8. Hall, H. and S.-O. Ogren. Effects of antidepressant drugs on different receptors in the brain. *Eur J Pharmacol* **70:** 393–407, 1981.
9. Hingtgen, J. N. and M. H. Aprison. Behavioral depression in pigeons following L-tryptophan administration. *Life Sci* **16:** 1471–1476, 1975.
10. Hollister, L. E. Tricyclic antidepressants. *N Engl J Med* **299:** 1106–1109 and 1168–1172, 1978.
11. Koe, K. B. Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. *J Pharmacol Exp Ther* **199:** 649–661, 1976.
12. Leander, J. D. Rate-dependent effects of drugs. II. Effects of some major tranquilizers on multiple fixed-ratio, fixed-interval schedule performance. *J Pharmacol Exp Ther* **193:** 689–700, 1975.
13. Leander, J. D. Drug effects on multiple and alternating mixed-schedule performance. *J Pharmacol Exp Ther* **218:** 728–733, 1981.
14. Leander, J. D. Antagonism of oxotremorine-induced behavioral suppression by antimuscarinic drugs. *Psychopharmacology (Berlin)* **75:** 5–8, 1981.
15. McKearney, J. W. The relative effects of *d*-amphetamine, imipramine and harmaline on tetrabenazine suppression of schedule-controlled behavior in the rat. *J Pharmacol Exp Ther* **159:** 429–440, 1968.
16. McKearney, J. W. Effects of tricyclic antidepressant and anticholinergic drugs on fixed-interval responding in the squirrel monkey. *J Pharmacol Exp Ther* **222:** 215–219, 1982.
17. O'Donnell, J. M. and L. S. Seiden. Differential-reinforcement of low rate 72-second schedule: Selective effects of antidepressant drugs. *J Pharmacol Exp Ther* **224:** 80–88, 1983.
18. Ross, R. B. and A. L. Renyi. Tricyclic antidepressant agents. I. Comparison of the inhibition of the uptake of ^{3}H-noradrenaline and ^{14}C-5-hydroxytryptamine in slices and crude synaptosome preparations of the midbrain-hypothalamus region of the rat brain. *Acta Pharmacol Toxicol* **36:** 382–394, 1975.
19. Ross, R. B. and A. L. Renyi. Tricyclic antidepressant agents. II. Effects of oral administration on the uptake of ^{3}H-noradrenaline and ^{14}C-5-hydroxytryptamine in slices of the midbrain-hypothalamus region of the rat brain. *Acta Pharmacol Toxicol* **36:** 395–408, 1975.

20. Smith, C. B. Effects of *d*-amphetamine upon operant behavior of pigeons: Enhancement by reserpine. *J Pharmacol Exp Ther* **146:** 167–174, 1964.
21. Vaillant, G. E. A comparison of chlorpromazine and imipramine on behavior of the pigeon. *J Pharmacol Exp Ther* **146:** 337–384, 1964.
22. Vaillant, G. E. A comparison of antagonists of physostigmine-induced suppression of behavior. *J Pharmacol Exp Ther* **157:** 636–648, 1967.
23. Wong, D. T. and F. P. Bymaster. Fluoxetine and nisoxetine selectively protect the uptake sites of 5-hydroxytryptamine and norepinephrine from neurotoxicity of *p*-chloroamphetamine and 6-hydroxydopamine, *in vivo*. *Res Commun Chem Pathol Pharmacol* **15:** 221–231, 1976.
24. Wong, D. T., F. P. Bymaster, J. S. Horng, K. L. Hauser and B. B. Molloy. A selective inhibitor of serotonin uptake: Lilly 110140, 3-(*p*-trifluoromethylphenoxy)-N-methyl-3-phenyl propylamine. *Life Sci* **15:** 471–479, 1974.
25. Wong, D. T., J. S. Horng and F. P. Bymaster. *dl*-N-Methyl-3-(p-methoxyphenoxy)-3-phenylpropylamine hydrochloride. Lilly 94939, a potent inhibitor for uptake of norepinephrine into rat brain synaptosomes and heart. *Life Sci* **17:** 755–760, 1975.
26. Ziegler, V. E., J. T. Biggs, L. T. Wylie, S. H. Rosen, D. J. Hawf and W. H. Coryell. Doxepin kinetics. *Clin Pharmacol Ther* **23:** 573–579, 1978.