Pharmacology Biochemistry and Behavior, Vol. 2, pp. 45–54. ANKHO International Inc., 1974. Printed in the U.S.A.

Involvement of Muscarinic and Nicotinic Receptors in Behavioral Tolerance to DFP[1]

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(Received 7 September 1973)

OVERSTREET, D. H., R. W. RUSSELL, B. J. VASQUEZ AND F. W. DALGLISH. *Involvement of muscarinic and nicotinic receptors in behavioral tolerance to DFP.* PHARMAC. BIOCHEM. BEHAV. 2(1) 45–54, 1974. – Effects of various cholinergic agents on the free operant responding and single alternation behavior of rats were examined following two regimens of chronic treatment with diisopropylfluorophosphate (DFP), an irreversible anticholinesterase, which lowered brain cholinesterase to 45% and 30% of normal, respectively. Reduction to 45% produced no observable changes in behavior; reduction to 30% gave rise to a decrease in the number of reinforced responses and an increase in the number of nonreinforced responses. Tolerance for the former measure developed within 10 days, whereas tolerance for the latter was not observed. Subsequent challenges were carried out using anticholinesterase agents, and muscarinic and nicotinic agonists and antagonists. The results suggest that the sensitivity of both muscarinic and nicotinic receptors to acetylcholine may be reduced during chronic treatment with DFP, but that muscarinic receptors may be more labile than nicotinic receptors. It is hypothesized that this reduction in sensitivity is one mechanism underlying the development of behavioral tolerance to DFP.

Anticholinesterases Behavioral tolerance Muscarinic receptors Nicotinic receptors Free operant responding Single alternation behavior

SINCE we [37] first reported rats tolerant to the anticholinesterase, diisopropylfluorophosphate (DFP), were more sensitive to the response-suppressing effects of atropine, a centrally acting antimuscarinic agent, than were control animals, there have appeared a number of reports which have confirmed and extended these findings. The water intake of DFP-treated rats was suppressed to a greater extent by scopolamine, another centrally acting antimuscarinic agent, than was that of normal rats [7]. Conversely, both the eating behavior and the fixed ratio responding of rats chronically treated with DFP were significantly less affected by the muscarinic agonist, pilocarpine, than were the corresponding behaviors of control rats [28,35]. The fact that both pilocarpine and atropine act directly upon the muscarinic receptors for acetylcholine (ACh) suggested that a change in the number or the conformation of these receptors might be a likely mechanism underlying the development of tolerance to DFP [28, 35, 37].

It was also reported [35] that DFP-treated animals were not cross-tolerant to the reversible anticholinesterases, physostigmine and neostigmine, a result which appears to be inconsistent with the receptor change hypothesis. However, this lack of cross-tolerance may occur because of a differential involvement of muscarinic and nicotinic receptors in tolerance development: administration of anticholinesterases increases ACh content [21, 35, 42] but the relative sensitivity of muscarinic and nicotinic receptors may be affected differentially by the build-up of ACh during chronic DFP treatment.

The present experiments were designed to examine this hypothesis by studying behavioral effects in DFP-treated and control rats of challenges with cholinergic agents which act upon nicotinic receptors and others which act on muscarinic receptors. Effects on free operant responding and single alternation behavior were examined. All the findings of the present experiments are consistent with the conclusion that a reduction in the sensitivity of muscarinic receptors may occur with smaller decreases in brain ChE activity than are required to produce a reduction in the sensitivity of nicotinic receptors.

A second purpose of the present investigations was to examine in more detail the characteristics of tolerance to DFP in a single alternation task in order to compare these with the characteristics of tolerance development to DFP in other behavioral tasks, e.g., eating, drinking, operant responding [28, 35, 37, 38, 47]. The alternation task seemed particularly appropriate for comparing the time characteristics of tolerance development because it permitted the

[1]This research was supported by Grant MH 18788 from the National Institute of Mental Health to Roger W. Russell and formed part of a dissertation submitted by the first author to the School of Biological Sciences, University of California, Irvine, as partial fulfillment for the degree, Doctor of Philospphy.

[2]Supported by PHS predoctoral fellowship 48981-02.

examination of several responses having different consequences but elicited in the same general environment: in this situation the animal must learn to press a bar for water reinforcement on one trial (S^+ responses) and to inhibit responses to the same stimulus on alternate trials (S^- responses) [19]. On the basis of earlier work [38, 47] and because of the suspected involvement of the cholinergic system in behavioral inhibition [3, 4, 45, 46], it was predicted that tolerance to DFP would occur more rapidly for the S^+ than for the S^- responses. This hypothesis was completely supported by the results of the present studies.

METHOD

Animals

The animals for each experiment consisted of separate groups of 16 male Sprague-Dawley (Simonsen) rats. They were approximately 90 days old and weighed approximately 450 g at the start of the experiments. An experimental group receiving chronic DFP treatment and a control group receiving chronic Arachis Oil treatment were formed by the random assignment of 8 animals to each of the two groups for each experiment.

Apparatus

Four standard operant chambers, 25 x 23.75 x 18.75 cm. which were housed in ventilated cabinets, were used. Programming equipment (BRS) delivered a drop of water (0.05 ml) with each appropriate press of a lever mounted on the left side of the chamber. The programming equipment also recorded the totals during the 1-hr session for the following measures: total responses for the free operant behavior in Experiment 1; and trials, S^+'s, S^-'s, responses during intertrial intervals (ITI's), and correct alternations (defined as an S^+ following the absence of an S^-) for single alternation behavior in Experiment 2. An Esterline-Angus event recorder was used to obtain a trial by trial record of the animal's single alternation performance, while a Massey-Dickinson print-out counter, which was activated every 30 sec, provided a cumulative record of the free operant responses.

Research Design

The rats were randomly assigned to the experimental and control groups. They were housed 4 to a cage, with 2 from each group in each cage. The design was balanced so that there was an equal number of experimental and control animals in each of the 4 operant chambers.

The basic research design for each experiment consisted of three phrases: (1) establishment of behavioral baselines; (2) chronic treatment with DFP or Arachis Oil; (3) acute treatment with the challenge agents. A 4 x 4 latin square design [10] was used in order to obtain dose-response data for most of the challenges. The 4 treatments were: three doses of a particular challenge agent and saline, the vehicle for all the agents.

Procedure

For free operant behavior the animals were maintained on a 23-hr deprivation schedule. In Phase 1 1-hr daily test sessions were continued until each animal's response rate over a 5-day period showed less than 10% variability. In Phase 2 one group of animals was subjected to a chronic regimen of DFP treatments consisting of administrations at 3-day intervals which lowered brain ChE activity to 46% of normal as determined by biochemical assays[9] at the conclusion of the experiment. The second group were treated identically, except with Arachis Oil rather than DFP. All injections occurred immediately after a behavioral test session. Phase 3 began after 8 administrations of DFP or Arachis Oil. Challenge agents were administered intraperitoneally (i.p.) prior to the test session which occurred 23 hr after an injection of DFP. Previous work from our laboratory [7,37] had provided dose-response data for atropine and scopolamine from which optimal challenge doses of these agents could be selected. Doses of mecamylamine were selected as intermediate between the low (0.5 mg/kg) and high (30.0 mg/kg) doses reported by other investigators to affect behavior [12, 15, 26, 27, 40]. When all the challenges were completed, the animals were sacrificed after their final behavioral test session. The rats were sacrificed by decapitation; whole brains were rapidly removed and homogenized in 10 ml of 10% sucrose. The ChE activity of these homogenates was determined colorimetrically [9]. The protein in each sample was determined by the standard Lowry method [23].

In the alternation situation the animals were maintained on a 23-hr deprivation schedule in Phase 1 until all animals met the baseline criterion of the establishment of 90% correct alterations, i.e., the ratio of correct alternations to S^+'s, for three consecutive days. During the alternation task each S^+ trial lasted 5 sec but was terminated by a bar press; each S^- trial lasted for 5 sec, with responses having no programmed consequences; and each intertrial interval lasted for 5 sec, with ITI's postponing the onset of the next trial for 5 sec. All animals received a 10-min water supplement following each session. Phase 2 consisted of chronic treatment with a standard regimen of DFP [14,38]: an initial injection of 1.0 mg/kg was followed by 0.5 mg/kg booster doses at 3-day intervals. The first challenge agent was administered after 20 injections of DFP or Arachis Oil, when it was apparent that tolerance was not developing in the S^- measure.

RESULTS

For determining the effects of the various pharamcological treatments on free operant behavior each animal was used as its own control. During Phase 2, the chronic injection phase, each animal's daily response output was expressed as a percentage of his preinjection baseline. Significance of the DFP treatment was analyzed by comparing the percentage baselines of the experimental and control groups by means of the Mann-Whitney U Test [41]. In analyzing the data from Phase 3, each animal's response output on a challenge session was expressed as a ratio of the response output on the immediately preceding day. This procedure was followed in order to eliminate effects of any long-term fluctuation in baseline performance. The significance of the challenge treatments was determined by Mann-Whitney U Tests and Friedman Two-Way Analyses of Variance [41].

In the analysis of alternation performance S^+'s, S^-'s, ITI's and % Correct Alternations were examined. Deviations from the pre-DFP baselines were used to determine the effects of the various pharmacological treatments.

Effects of Chronic Treatment

Free operant behavior. There was no significant differ-

ence between the performances of the two groups during the pre-DFP baseline period (U=25, p NS), i.e., the groups were comparable initially. Similar tests on the percentage baselines also showed no significant differences between the groups at any time during the chronic DFP treatment phase. Analyses of ChE activity in whole brain homogenates of 4 control and all DFP-treated rats at the end of the experiment showed that the median per cent of control levels for the DFP-treated rats was 46.0%.

Single alternation behavior. At the start of the chronic treatment with DFP or Arachis Oil, the two groups were comparable in single alternation performance as indicated by the following baseline measures: S^+ = 196, 191; S^- = 17, 14; ITI = 46, 61; % Correct Alternations = 94.9, 95.8 for the control and DFP-treated groups, respectively. Mann-Whitney U Tests revealed no significant differences in the baselines of the two groups.

Analyses of the median deviations from baseline during the chronic treatment phase are summarized in Table 1. Examination of the table reveals certain basic features: (a) the DFP-treated group made significantly fewer S^+ responses after the first treatment, but the effects diminished with succeeding injections so that by the fourth treatment there was no longer a significant difference between the control and DFP-treated animals, i.e., tolerance developed within 10 days; (b) the number of S^- responses for the DFP-treated group remained significantly above baseline levels, but tended to decrease in the control group, i.e., there was no evidence of tolerance development; (c) at no time were the differences in ITI's between control and DFP-treated groups significant, both decreasing as treatments continued; (d) DFP-treated animals remained significantly below while controls were generally above their baseline % Correct Alternations, again indicating that tolerance had not developed.

Effects of Challenge Agents

Intragroup Effects. The effects of the challenge agents on total response output during the 1-hr test session for free operant behavior are reported in Table 2 in the order in which the challenges occurred. Examination of the columns of ratios for each groups suggests that most challenge agents produced dose-dependent decreases in responding. Friedman tests established that all were in fact statistically significant except for carbachol and nicotine in the DFP-treated group.

Tables 3 and 4 summarize the effects of the challenge agents on the 4 measures of single alternation performance, again in the order in which they were administered. Results of Friedman analyses of variance justify the following statements: all challenges, except for carbachol in the control group, produced a dose-dependent decrease in the S^+'s; the pattern of effects on S^-'s was more complex, pilocarpine reducing performance in the control rats, but increasing it

TABLE 1

EFFECTS OF CHRONIC TREATMENT WITH DFP OR ARACHIS OIL (Ao) ON VARIOUS MEASURES OF SINGLE ALTERNATION BEHAVIOR IN MALE RATS

Number of DFP Treatments	Deviations from Baseline (Medians, N = 8) S+ AO	S+ DFP	S− AO	S− DFP	ITI AO	ITI DFP	% AO	% DFP
1	+ 4.0	−75.5*	−3.0	+15.0*	−16.0	−23.0	+1.3	−11.0*
2	+ 3.0	−36.0*	+1.0	+15.5*	− 7.5	−17.0	−1.5	− 6.0*
3	+ 8.0	− 7.0*	−3.0	+14.5*	− 6.5	+16.0	+1.2	− 3.2*
4	+ 0.5	−17.0	−4.5	+12.0*	− 9.0	−12.5	+0.6	− 6.2*
5	− 3.5	−16.5	+4.0	+28.0	− 8.5	−21.5	+0.2	− 5.9
6	−19.0	−34.0	+0.5	+17.0*	−37.5	−10.0	−0.8	− 8.4*
7	−10.0	−24.0	−4.5	+16.5*	− 9.5	− 6.0	+0.2	− 5.7*
8	− 4.5	−16.0	−5.5	+13.5*	− 9.5	−15.5	−0.6	− 5.8*
9	− 2.5	−33.0	−3.5	+20.5*	−24.0	−20.5	+1.6	− 6.0*
10	+ 4.0	−28.0	−9.5	+16.0*	− 9.5	+ 2.5	+3.2	− 6.6*
11	− 6.5	−18.5	−4.0	+15.5*	−10.5	−11.5	+0.3	− 5.6*
12	− 5.5	−19.0	−8.0	+17.5*	−14.5	−15.5	+2.3	− 6.1*
13	− 3.5	−18.0	−4.0	+ 7.5*	−16.0	−35.0	+0.8	− 3.2*
14	− 5.5	−11.5	−4.5	+17.0*	−14.0	− 7.0	+0.7	− 8.2*
15	− 6.5	−13.0	−6.0	+17.5*	−15.5	−33.5	+1.7	− 5.7*
16	− 6.5	−13.5	−6.0	+12.0*	−14.5	−30.5	+1.1	− 5.0*
17	+10.5	−17.5	−5.5	+19.5*	−14.5	−31.0	+0.9	− 5.8*
18	+ 9.0	−17.5	−4.5	+16.5*	−17.0	−26.5	+1.1	− 7.5*
19	+ 0.5	−11.0	−9.0	+ 8.5*	−13.5	−28.0	+1.6	− 2.3*

*Significantly different ($p<0.05$) from control subjects by Mann-Whitney U Tests [41].

TABLE 2

EFFECTS OF CHALLENGE AGENTS ON FREE OPERANT RESPONDING OF CONTROL AND DFP-TREATED RATS

Challenge Agent*	Dose† (mg/kg)	Challenge/Pre-Challenge Ratios Control N = 8	DFP-Treated N = 8
Physostigmine (0)	0.0	96.0	91.9
	0.1	87.2	81.5
	0.2	55.2	73.5
	0.4	54.3	43.2
Neostigmine (0)	0.1	78.1	82.5
Carbachol (0)	0.0	97.2	101.1
	0.1	91.0	87.3
	0.2	83.5	96.8
	0.4	76.6	96.9‡
Pilocarpine (0)	0.0	89.4	93.5
	2.0	94.6	120.3
	4.0	71.5	112.2‡
	8.0	4.7	74.4‡
Methyl atropine (30)	8.0		
+ Pilocarpine (0)	8.0	30.9	70.2‡
Atropine (30)	8.0	58.7	48.8
	12.0	65.4	8.4‡
Methyl atropine (30)	8.0	72.8	72.9
	12.0	60.7	68.8
Nicotine (0)	0.0	100.1	93.7
	0.1	96.1	93.6
	0.2	81.1	93.6
	0.4	67.1	76.5
Mecamylamine (15)	0.0	87.2	91.9
	1.0	88.7	85.8
	2.0	72.8	84.1
	4.0	66.1	72.9
Scopolamine (30)	1.0	39.8	4.9‡

*Number in parenthesis refers to the time of administration in min before the start of the behavioral session.

†Refers to the dose of the respective salt: sulfate for physostigmine, neostigmine, atropine and nicotine; chloride for carbachol; nitrate for methyl atropine; and hydrochloride for pilocarpine, mecamylamine and scopolamine.

‡Significantly different from controls at the $p<0.05$ level by Mann-Whitney U Tests [41].

in the DFP-treated rats; atropine and mecamylamine increased S^-'s in both groups; with the exception of atropine and nicotine the challenge agents produced a dose-dependent decrease in ITI's; finally, all challenge agents other than carbachol reduced the 5% Correct Alternations in a dose-related manner.

Intergroup effects. Reference to Table 2 indicates that the only significant intergroup differences in free operant responding occurred as a result of challenges with agents which act upon muscarinic receptors: both cholinomimetics, i.e., carbachol and pilocarpine, depressed the responding of DFP-treated animals to a lesser extent than that of the controls; conversely, the centrally acting antimuscarinics, i.e., atropine and scopolamine, depressed the responding of DFP-treated animals to a greater extent than that of controls. When the peripheral effects of pilocarpine were blocked by prior injection of methyl atropine, the behavior of the DFP-treated rats was still significantly less affected than was that of controls. None of the other agents produced significant intergroup effects.

Significant intergroup differences in S^+'s occurred as a result of challenges with nicotine, pilocarpine, and atropine (Table 3). Both the nicotinic and muscarinic agonists depressed the responding of the DFP-treated animals to a lesser extent than that of control animals. In contrast, atropine, a centrally acting muscarinic antagonist, depressed the responding of DFP-treated animals to a greater extent than that of controls. When the peripheral effects of pilocarpine were blocked by prior treatment with methyl atropine the number of S^+'s of the DFP-treated group were still less depressed than were those of the control group.

The differences between the control and DFP-treated animals for ITI's were never significant (Table 4). The large variability among individual animals may have accounted for this lack of significant intergroup differences.

Interpretation of intergroup effects for S^-'s and % Correct Alternations is complicated by the fact that significant differences were observed with saline challenges even after extended chronic treatment with DFP. Such intergroup differences were to be expected because of the fact stated earlier that tolerance had not developed for these two measures when the challenges began.

Time-response effects. Because a number of the challenge agents produced an immediate effect upon the behavior of both groups but were metabolized within the 1-hr test session, time-response analyses of the drug effects are essential to provide information about possible intergroup differences which could be masked by analyses of total response output only. Such analyses can be rpesented most clearly by graphic means. (Mechanical failures prevented time-response analyses for physostigmine and neostigmine, but the required data for all other challenges were complete.)

Examination of Fig. 1 indicates that the responding of both groups ceased following the higher doses of carbachol, but that responding started again in the DFP-treated animals sooner than in the controls. Also apparent from the figure are the significant dose-response effects of carbachol in the DFP-treated rats. Recovery from all carbachol treatments was sufficiently rapid that the DFP-treated group was able to recover and attain a normal response output within the session.

Figure 2 shows the subsensitivity of the DFP-treated animals to pilocarpine. Particularly striking is the near superimposability of the DFP + 8.0 curve with the Arachis Oil +

TABLE 3

EFFECTS OF CHALLENGE AGENTS ON S+ AND S− RESPONSES OF CONTROL AND DFP–TREATED RATS

Challenge Agent*	Dose† (mg/kg)	Deviations from Baseline (Medians, N = 8) S+ AO	S+ DFP	S− AO	S− DFP
5					
Carbachol (0)	0.0	− 25.5	− 26.5	− 4.5	+11.0‡
	0.1	− 60.0	− 52.0	+ 1.0	+17.0‡
	0.2	− 63.0	− 49.0	− 2.0	+15.0‡
	0.4	−149.5	− 92.0	− 4.0	+ 1.5
Pilocarpine (0)	0.0	− 10.0	− 14.0	− 4.5	+11.5‡
	0.0	− 90.5	− 39.5	− 0.5	+25.5
	4.0	−143.0	− 51.5‡	−11.5	+20.5
	8.0	−195.5	−170.5‡	−14.0	− 3.0
Methyl atropine (30)	8.0				
+ Pilocarpine (0)	8.0	−114.0	− 60.0‡	− 8.0	+27.5‡
Nicotine (0)	0.0	0.0	− 14.5	− 1.5	+ 3.5
	0.1	− 38.5	− 18.0	+ 1.0	+11.5‡
	0.2	− 68.0	− 32.5‡	+ 2.5	+16.0‡
	0.4	− 85.5	− 59.5‡	+ 2.0	+ 4.0
Mecamylamine (15)	0.0	− 7.5	− 8.0	− 2.0	+ 6.0‡
	1.0	+ 1.5	− 18.5	− 5.0	− 6.5
	2.0	− 12.0	− 15.0	+ 3.0	+ 5.5
	4.0	− 47.0	− 19.5	+ 8.0	+15.5
	8.0	− 92.5	− 55.0	+ 7.5	+32.0‡
Atropine (30)	0.0	+ 2.0	+ 3.5	− 2.5	+ 2.0
	2.0	− 16.0	− 18.0	+ 1.5	+17.5‡
	4.0	− 35.0	− 52.0	+ 3.0	+42.0‡
	8.0	− 92.5	−137.0‡	+13.5	+19.5
Methyl atropine (30)	8.0	+ 5.0	+ 2.5	− 3.0	+ 4.5
Physostigmine (0)	0.4	−114.0	−164.0	− 4.0	− 1.5

*Number in parentheses refers to the time of administration in min before the start of the behavioral session.

†Refers to the dose of the respective salt; see Table 2.

‡Significantly different from controls at the $p<0.05$ level by Mann-Whitney U Tests [41].

4.0 curve, suggesting that pilocarpine must be increased by a factor of 2 to obtain the same behavioral effect in the DFP-treated rats.

The curves in Fig. 3 clearly differentiate central and peripheral effects of pilocarpine. Comparison of the two curves showing effects of pilocarpine with those showing effects of methyl atropine + pilocarpine indicates that a slowing of the response rate is due primarily to the central effects of pilocarpine, while a combination of the central and peripheral effects of this agent produce complete cessation of behavior. Thus, DFP-treated animals both started responding sooner after the injection and responded at a higher rate than did the controls, indicating that they were subsensitive to both the peripheral and central effects of pilocarpine.

The time-response data for nicotine, presented in Fig. 4, show that nicotine had analogous effects in the two groups,

TABLE 4

EFFECTS OF CHALLENGE AGENTS ON ITI RESPONSES AND % CORRECT ALTERNATIONS OF CONTROL AND DFP-TREATED RATS

Challenge Agent*	Dose† (mg/kg)	Deviations from Baseline (Medians, N = 8) ITI AO	ITI DFP	% AO	% DFP
Carbachol (0)	0.0	−12.0	−33.0	+ 0.4	− 6.5‡
	0.1	−12.0	−37.0	− 2.7	− 9.3‡
	0.2	−24.5	−43.0	− 0.9	− 7.1‡
	0.4	−44.5	−56.0	− 2.3	− 6.3
Pilocarpine (0)	0.0	−16.5	−10.5	+ 0.8	− 4.8‡
	2.0	−17.5	−19.5	− 7.9	−12.8
	4.0	−26.0	−22.0	−14.4	−12.0
	8.0	−50.5	−34.0	−34.8	−10.6
Methyl atropine (30)	8.0	−45.5	−16.0	− 7.1	−10.9
+ Pilocarpine (0)	8.0				
Nicotine (0)	0.0	− 5.0	−27.5	+ 0.6	− 2.8‡
	0.1	−16.5	− 2.5	− 2.4	− 4.8
	0.2	−17.5	+12.0	− 2.0	− 9.2‡
	0.4	−24.5	−12.5	− 5.8	− 6.6
Mecamylamine (15)	0.1	−11.0	−25.0	0.0	− 3.5‡
	1.0	−24.5	−42.0	+ 1.0	− 2.1
	2.0	−27.5	−31.5	− 1.5	− 4.3
	4.0	−48.0	−49.0	− 5.5	− 4.7
	8.0	−41.5	−40.0	− 5.7	−15.3
Atropine (3)	0.0	−18.0	−28.5	+ 1.0	− 2.9
	2.0	−18.5	− 9.5	− 2.8	− 8.1‡
	4.0	−16.5	− 7.5	− 2.1	−17.6‡
	8.0	−22.5	− 9.5	−12.7	−43.9‡
Methyl atropine (30)	8.0	−20.0	−47.0	− 1.1	− 3.4
Physostigmine (0)	0.4	−28.0	−54.0	− 6.7	−18.0

*Number in parenthesis refers to the time of administration in min before the start of the behavioral session.

†Refers to the dose of the respective salt; see Table 2.

‡Significantly different from controls at the $p<0.05$ level by Mann-Whitney U Tests [41].

indicating that there were no significant intergroup differences as a result of challenging with this agent. Also apparent from the figure are the dose-response effects of nicotine in both groups as reflected in the systematic shift of the curves along the time axes.

Analyses of the data from the Esterline-Angus event recorder revealed that the time-response effects of the short-acting challenge agents in this task were comparable to those in the free operant task: DFP-treated animals given nicotine, pilocarpine, and carbachol recovered sooner than did the control rats.

DISCUSSION

Tolerance Development

The DFP treatment regimen used in the first experiment lowered brain ChE activity to 46% of normal and no consequent behavioral effects were noted, as could be

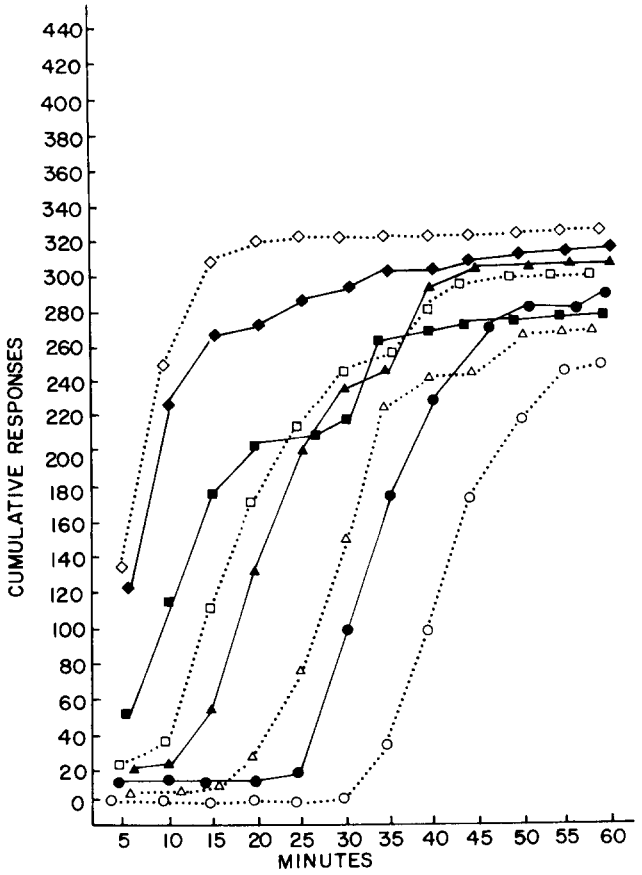

FIG. 1. Time-Response Effects of Carbachol on the Free-Operant Responding of Control and DFP-treated Rats. The carbachol was administered i.p. immediately before the behavioral session. Each curve represents the median for 8 animals. ◇ • • • ◇ = AO + 0.0, ◆——◆ = DFP + 0.0, □ • • • □ = AO + 0.1, ■——■ = DFP + 0.1, △ • • • △ = AO + 0.2, ▲——▲ = DFP + 0.2, ○ • • • ○ = AO + 0.4, ●——● = DFP + 0.4.

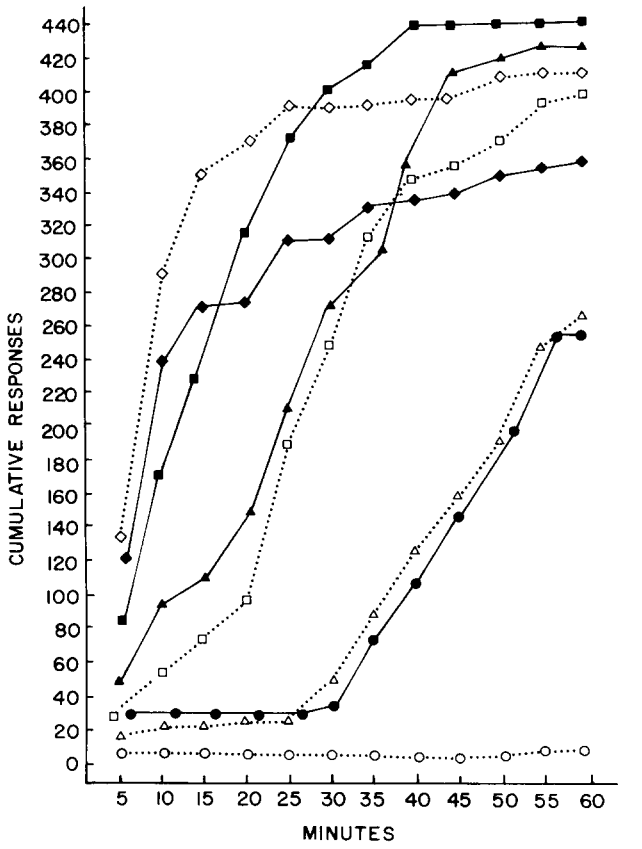

FIG. 2. Time-Response Effects of Pilocarpine on the Free-Operant Responding of control and DFP-treated Rats. The pilocarpine was administered i.p. immediately before the behavioral session. Each curve represents the median for 8 animals. ◇ • • • ◇ = AO + 0.0, ◆——◆ = DFP + 0.0, □ • • • □ = AO + 2.0, ■——■ = DFP + 2.0, △ • • • △ = AO + 4.0, ▲——▲ = DFP + 4.0, ○ • • • ○ = AO + 8.0, ●——● = DFP + 8.0.

predicted on the basis of earlier findings that brain ChE activity must be acutely lowered to below a threshold at 45% of normal before behavioral effects are observed [13, 33, 34, 39]. This lack of initial effect meant that tolerance to DFP could not be observed by changes in overt behavior [37]. We have interpreted such results in terms of a two process model of tolerance development: if the rate of reduction of ChE activity is slower than the rate of the process underlying tolerance to DFP, the two antagonistic processes may counterbalance each other so that no behavioral effects are observed [7]. That some tolerance did in fact develop in the present DFP-treated animals is evidenced by the significant intergroup differences during the later challenges with atropine and pilocarpine, thus confirming our earlier observations of other behavior patterns [7,28].

Acute DFP treatment exhibited three major effects upon the single alternation behavior of the rats in the second study: the number of S^+ responses was significantly depressed; the S^-'s were significantly elevated; and the ITI responses were unaffected. Tolerance to DFP for the S^+ measure developed within 10 days of chronic treatment. This finding is similar to results obtained earlier in our laboratory: behavioral tolerance to DFP developed within 10 days in a variety of behavioral measures [37, 38, 47], including a discrete trial operant response which was very similar to the S^+ measure in the present study and was affected in an analogous manner.

On the other hand, two behavioral measures, S^-'s and % Correct Alternations, did not return to pretreatment levels. This finding of a lack of complete tolerance development to DFP for these two measures of inhibitory responding may help to clarify some earlier work. For example, in one study [32] animals with chronically reduced ChE activity had difficulty in inhibiting nonreinforced responses. This finding may have been because the development of tolerance was incomplete. This interpretation may also be pertinent to similar results reported by investigators measuring other behavior patterns [1,39].

Receptor Changes

The major purpose of the present experiments was to study the possible involvements of muscarinic and nicotinic receptors in the development of tolerance to DFP. The effects of challenges with muscarinic agents were entirely

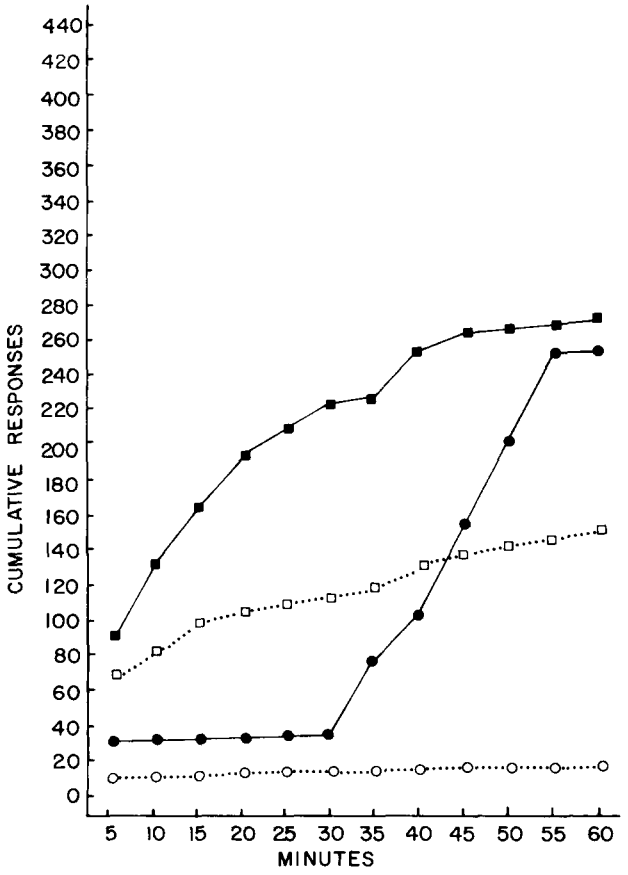

FIG. 3. Comparison of Time-Response Effects of Pilocarpine Alone with a Combination of Methyl Atropine and Pilocarpine on the Free-Operant Responding of Control and DFP-treated Rats. Methyl atropine and pilocarpine, respectively, were injected 30 min and immediately before the behavioral session. Each curve represents the median for 8 animals. □ • • •□ = AO + Methyl Atropine + Pilocarpine; ■——■ = DFP + Methyl Atropine + Pilocarpine; ○ • • •○ = AO + Pilocarpine; ●——● = DFP + Pilocarpine. The dose for each was 8.0 mg/kg.

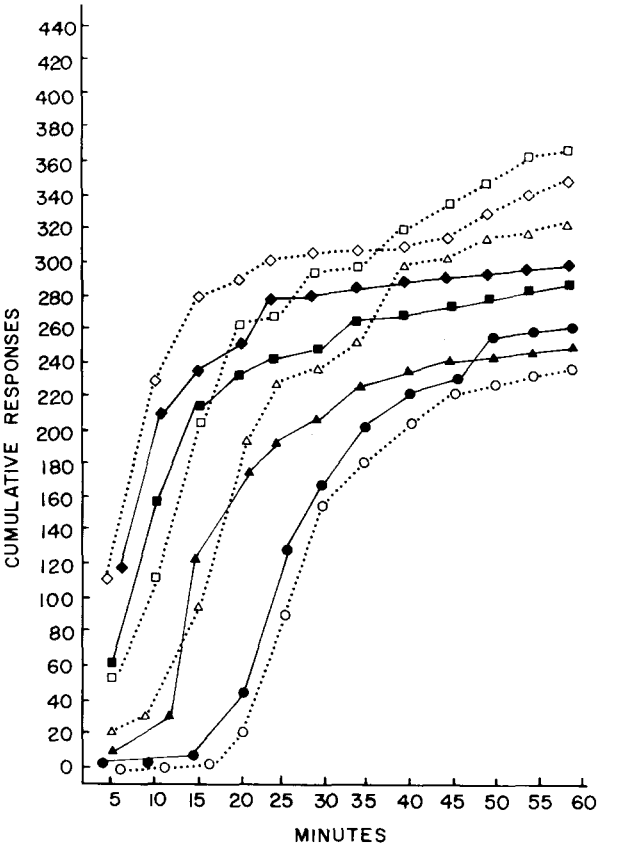

FIG. 4. Time-Response Effects of Nicotine on the Free-Operant Responding of Control and DFP-treated Rats. The nicotine was injected i.p. immediately before the behavioral session. Each curve represents the median for 8 animals. ◇ • • •◇ = AO + 0.0, ◆——◆ = DFP + 0.0, □ • • •□ = AO + 0.1, ■——■ = DFP + 0.1, △ • • •△ = AO + 0.2, ▲——▲ = DFP + 0.2, ○ • • •○ = AO + 0.4, ● ● = DFP + 0.4.

consistent with previous work in this field: animals chronically treated with DFP were subsensitive to pilocarpine and carbachol and supersensitive to atropine [28, 35, 37]. These findings may be interpreted to support the hypothesis that the sensitivity of muscarinic receptors to ACh is altered during chronic DFP treatment.

Changes in the sensitivity of muscarinic receptors were observed in both experiments, i.e., when ChE activity levels were at 46% and 30% of normal respectively. In contrast, the sensitivity of nicotinic receptors was altered with ChE at 30% of normal, but not at 45%. This suggests that the latter are more resistant to change than are the former.

The results of the present experiments involve a situation in which decreases in ChE activity give rise to increases in ACh content. The hypotheses of differential changes in receptor sensitivity during tolerance development, i.e., lability of muscarinic receptors and relative stability of nicotinic receptors, has received support from experiments in which ACh content is decreased by denervation of autonomic ganglia: under these circumstances the sensitivity of nicotinic receptors remained the same while that of muscarinic receptors was greatly enhanced, as measured electrophysiologically [18,43].

Mecamylamine, a centrally acting nicotine antagonist [44], did not produce a differential effect on the behavior of the control and DFP-treated rats in either experiment, even though nicotine itself produced such an effect in the second experiment. This finding contrasts with that found after challenge with the muscarinic agents: both the agonist (pilocarpine) and the antagonist (atropine) resulted in differential effects on the behavior of the two groups in each experiment. Although the doses of mecamylamine were high enough to result in behavioral deficits in both groups in the present studies and higher than those used in some other studies [26, 27, 40], it is possible that differential effects would not occur until even higher doses were used. Only further studies with this agent can answer this question. In any event the lack of differential effects at the doses of mecamylamine used in the present studies may be further evidence to support the hypothesis that the nicotine receptors are more resistant to change than are the muscarinic receptors.

The results of the present and other recent studies from

our laboratory [7, 28, 35, 37] clearly establish that a reduction in the sensitivity of cholinergic receptors may be one mechanism underlying the development of tolerance to DFP. Only further studies can establish whether this reduced sensitivity is the result of an alteration in the conformation of the receptors [20, 30, 31], a reduction in their numbers [6], or a change in the metabolism of the postsynaptic cell at some stage beyond the receptor [11].

Results of the present studies suggest that during chronic DFP treatment changes in muscarinic receptors may occur in both peripheral and central tissues. For example, the quaternary cholinomimetic, carbachol, whose primary effects are peripheral [22], affected the behavior of the DFP-treated rats to a lesser extent than that of control rats. This result supports the findings of other investigators who have reported a subsensitivity to carbachol and pilocarpine in isolated peripheral tissues of animals chronically treated with anticholinesterase agents [2, 5, 11, 16, 29]. Changes in central muscarinic sensitivity must also have occurred. When the peripheral effects of pilocarpine, a tertiary cholinomimetic, were blocked by methyl atropine, the behavior of the DFP-treated animals was still significantly less affected than was that of controls.

Cross-tolerance

In the original design of the single alternation study challenge with physostigmine was not included. However, when the DFP-treated animals appeared to be subsensitive to both nicotine and pilocarpine, it was decided to challenge with physostigmine. The lack of any cross-tolerance between physostigmine and DFP confirms earlier results [35] and cannot be attributed to a lack of alteration of nicotinic receptors. Tolerance to anticholinesterase agents is only partial because there are several instances of an increased sensitivity to one anticholinesterase following chronic treatment with another [17, 24, 25]. The lack of cross-tolerance between physostigmine and DFP indicates that these two anticholinesterases may differ in their mechanisms of action. The demonstration of several isozymes of AChE [8] suggests that DFP and physostigmine may inhibit different subpopulations of the enzyme. Thus, physostigmine may disrupt behavior by inhibiting an isozyme of ChE that is resistant to DFP.

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