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MUSCARINIC RECEPTOR SUBSENSITIVITY IN THE LONGITUDINAL MUSCLE OF THE RAT ILEUM FOLLOWING CHRONIC ANTICHOLINESTERASE TREATMENT WITH DIISOPROPYLFLUOROPHOSPHATE*

FREDERICK J. EHLERT,† NORIO KOKKA‡ and ALAN S. FAIRHURST

Department of Medical Pharmacology and Therapeutics, University of California at Irvine, Irvine, CA 92717, U.S.A.

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Abstract—Chronic administration of diisopropylfluorophosphate to rats at a dose regimen producing 70 per cent inhibition of ileal cholinesterase was accompanied by a decrease in the sensitivity of the isolated ileum to oxotremorine and carbachol. The same treatment produced an increase in the sensitivity of the ileum to acetylcholine during the first ten days of anticholinesterase treatment, but after ten days of treatment the sensitivity of the ileum to acetylcholine was reduced. When [^{3}H]quinuclidinyl benzilate binding was measured in ileal longitudinal muscle homogenates from anticholinesterase-treated rats, a decrease in binding was observed which was due primarily to an increase in the dissociation constant of [^{3}H]quinuclidinyl benzilate. Chronic diisopropylfluorophosphate treatment also increased the K_i of various cholinergic ligands as determined by competitive displacement of [^{3}H]quinuclidinyl benzilate binding. Scatchard analysis of agonist displacement of [^{3}H]quinuclidinyl benzilate binding revealed two agonist binding sites which were present in approximately equal concentrations. Chronic diisopropylfluorophosphate treatment caused an increase in the dissociation constant of the high affinity site but produced only small effects on the dissociation constant of the low affinity site and on the relative concentration of the two sites. These results indicate that the tolerance to chronic anticholinesterase treatment is, in part, a receptor-mediated phenomenon.

The development of tolerance after chronic administration of an organophosphorus cholinesterase (ChE) inhibitor was first described by Rider *et al.* [1]. Subsequently, several investigators demonstrated that chronic ChE inhibition causes postjunctional subsensitivity in various muscarinic cholinergically innervated tissues [2–7]. Since these latter studies showed that postjunctional mechanisms are involved in the development of tolerance to anti-ChE agents, decreases in acetylcholine receptor affinity [8, 9] and density [10] have been postulated as possible mechanisms of tolerance. In a study of the specific binding of the labelled cholinergic antagonist [^{3}H]quinuclidinyl benzilate (QNB) to rat brain (striatal) preparations, we have now shown [11] that chronic treatment of the rats with diisopropylfluorophosphate (DFP) produces a decrease in the concentration of [^{3}H]QNB binding sites, together with a decrease in the affinity of these muscarinic receptors for various cholinergic ligands. In that study we also showed that, following DFP treatment, striatal muscarinic receptors displayed a greater decrease in affinity to muscarinic agonists than to antagonists. Further, Scatchard analyses of oxotremorine inhibition of [^{3}H]QNB binding showed that DFP treatment produced a decrease in affinity of the high affinity site but not of the low affinity site. Thus, the tolerance to chronic DFP treatment is seen from these striatal experiments to be, at least in part, a receptor-mediated phenomenon.

Relatively little is known of the effects of chronic anti-ChE treatment on peripheral neuroreceptors, although Chang *et al.* [12] obtained direct evidence for receptor-mediated subsensitivity in peripheral nicotinic cholinergic receptors, as shown by a decrease in the concentration of α-bungaro toxin binding sites in the rat diaphragm following chronic ChE inhibition with neostigmine. The present report describes the effects of chronic DFP treatment on the binding of cholinergic ligands to the longitudinal muscle of the ileum, this tissue being utilized since both contractility and receptor radioligand binding can be assayed, permitting direct comparison of changes in contractile responsiveness and muscarinic receptor parameters. Our results indicate that chronic ChE inhibition causes changes in the affinity and number of peripheral muscarinic receptors, which generally parallel those changes produced in the central nervous system, and establish that the tolerance in the periphery to chronic ChE inhibition is, in part, mediated via changes in the muscarinic receptors.

METHODS

DFP treatment. Male albino Sprague–Dawley rats, weighing 200–250 g, were housed two or three per

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† Present address: Department of Pharmacology, College of Medicine, Arizona Health Science Centre, Tucson, AZ 85724, U.S.A.

‡ Present address: Department of Pharmacology, University of California, Los Angeles, Los Angeles, CA 90024, U.S.A.

cage in the laboratory with free access to food and water. Sustained inhibition of ChE was produced by subcutaneous injections of DFP dissolved in peanut oil. Two mg/kg DFP were administered initially, followed by maintenance doses of 1 mg/kg DFP on alternate days after day two. At various times DFP-treated rats were decapitated and the ileum was removed. Unless stated otherwise, all rats were killed 24 hr after the last injection of DFP. All comparisons were made with untreated controls since it was found in preliminary experiments that the administration of peanut oil alone had no effects on ileal ChE.

Cholinesterase activity measurements. For measurement of ChE activity, a 2 cm section of ileum was washed in saline, blotted on filter paper, weighed, and minced with scissors. The tissue was homogenized in 50 vol. of ice-cold 0.1 M Na^+–K^+ phosphate buffer, pH 8.0, in a Potter–Elvehjem homogenizer and rehomogenized on the Polytron at setting 5 for 15 sec to a final concentration of 20 mg wet wt/ml buffer. ChE activity was determined by the method of Ellman *et al.* [13] within 4 hr after the animals were killed. The activity of ChE was expressed as μmoles acetylthiocholine hydrolyzed min^{-1} mg^{-1} protein. Protein was determined by the method of Lowery *et al.* [14] using bovine serum albumin as a standard.

Isolated ileum experiments. The muscarinic activity of acetylcholine, oxotremorine and carbachol was studied on 2 cm sections of ileum mounted in an organ bath containing aerated Tyrode's solution at 37°. Isometric contractions were measured with a force displacement transducer and polygraph. The resting tension of the ileum was adjusted to a load of 0.5 g, and a 30 min equilibration period preceded the dose–contractile response measurements. The ED_{50} values agonists were derived from log–dose response plots covering at least five drug concentrations. Atropine antagonism of oxotremorine-induced contractions was examined in ilea from control and DFP-treated rats. After dose–response measurements were obtained for oxotremorine in the absence of atropine, the ileum was allowed to equilibrate for 30 min in the presence of 10 nM atropine. Following equilibration, dose–response measurements were repeated at higher concentrations of oxotremorine. The dose ratio of atropine was calculated as the ratio of the ED_{50} of oxotremorine in the presence of atropine divided by ED_{50} in the absence of atropine. The significance of the difference between the agonist ED_{50} values in control and DFP-treated rats was determined by Student's *t*-test on logarithmic transformation of the data.

Muscarinic receptor binding assays. Specific [^{3}H]QNB binding to the longitudinal muscle of the ileum was determined according to the procedure of Yamamura and Snyder [15]. Only the longitudinal muscle layer was utilized since we observed that 90 per cent of the specifically bound [^{3}H]QNB in the whole ileum was distributed in the longitudinal muscle. Similar results were obtained by Yamamura and Snyder [15] in the guinea pig ileum. Rat longitudinal muscle was obtained by supporting the whole ileum on a 2-ml pipet and gently rubbing away the outer longitudinal muscle layer with a cotton swab. This layer was then minced with scissors, homogenized on a Potter–Elvehjem homogenizer, and rehomogenized on the Polytron at setting No. 5 for 1 min to a final concentration of 20 mg wet wt/ml 50 mM KH_2PO_4 + Na_2HPO_4 (pH 7.4) buffer. One hundred-μl aliquots of this homogenate were used in triplicate determinations of binding. The final volume of phosphate buffer in the incubation tubes was 2 ml. Binding in the presence of 10^{-4} M oxotremorine was defined as nonspecific. For measurement of the competitive displacement of [^{3}H]QNB binding by various nonlabeled ligands, a final concentration of 0.8 nM [^{3}H]QNB was used. At this concentration, less than 5 per cent of the total [^{3}H]QNB was bound. Acetylcholine inhibition of [^{3}H]QNB binding was determined in the presence of 10^{-6} M physostigmine to prevent enzymatic hydrolysis of acetylcholine.

Analysis of binding data. The binding parameters were determined from the experimental data by nonlinear least squares regression analysis. Binding was assumed to obey the Langmuir Relationship:

$$B = \sum_{j=1}^{n} \frac{X N_j}{X + K_j}, \qquad \text{(a)}$$

where B is the concentration of bound [^{3}H]QNB, N is the concentration of binding sites, K is the dissociation constant of [^{3}H]QNB and n is the number of classes of binding sites. As discussed below, $n = 1$ for muscarinic antagonists and 2 for agonists. For measurement of the competitive displacement of [^{3}H]QNB binding by nonlabeled ligands, B is the percentage of [^{3}H]QNB displaced by a given concentration, X, of nonlabeled drug and K is the apparent dissociation constant of the nonlabeled drug. The least squares fit to equation (1) was determined by the Gauss Newton method [16].

The dissociation constants of nonlabeled ligands were derived from the apparent dissociation constants as follows:

$$K = K_A (1 + \{[^3H]QNB\}/K_{QNB}),$$

where K is the dissociation constant of the nonlabeled ligand, K_A is the apparent dissociation constant derived from the least squares fit to equation ', $\{[^3H]QNB\}$ is the concentration of [^{3}H]QNB used in the experiment and K_{QNB} is the dissociation constant for [^{3}H]QNB determined from separate experiments on control and DFP-treated rats. The K_i values of nonlabeled ligands were calculated in a similar manner:

$$K_i = IC_{50}/(1 + \{[^3H]QNB\}/K_{QNB}),$$

where IC_{50} is the concentration of nonlabeled drug that caused half-maximal displacement of [^{3}H]QNB binding.

Statistical tests of the significance of the difference between the ligand binding parameters of control and DFP-treated rats were made using Student's *t*-test on logarithmic transformations of the IC_{50} values and apparent dissociation constants.

Drugs and chemicals. [^{3}H]Quinuclidinyl benzilate (16 Ci/mmole) was obtained from the Amersham/Searle Corp., Arlington Heights, IL, and its radiochemical purity was checked by thin-layer chromatography in two solvent systems (ethanol–acetic acid–water, 60 : 30 : 10; *n*-butanol–water–

acetic acid, 60 : 20 : 10). DFP was obtained from the Aldrich Chemical Co., Milwaukee, WI; acetylcholine, acetylthiocholine, atropine, carbachol, dithiobisnitrobenzoic acid and physostigmine, from the Sigma Chemical Co., St. Louis, MO.; oxotremorine was provided by Dr. Donald Jenden, UCLA.

RESULTS

The initial injection of 2 mg/kg DFP produced signs of marked cholinergic stimulation characterized by diarrhea, tremor, salivation and lacrimation. Similar effects of reduced intensity were produced by injection of the maintenance dose of 1 mg/kg DFP on days 3 and 5. However, few or no signs of cholinergic stimulation were seen on days 7–9, indicating that tolerance had developed to DFP. This dose regimen of DFP produced a constant 70 per cent inhibition of ileal ChE activity as determined 24 hr after injection on days 1, 4, 10, 14, 20 and 26 of chronic DFP treatment.

The results of the dose–response experiments on the isolated ilea of control and DFP-treated rats are shown in Fig. 1 The ED_{50} of acetylcholine at 2 hr was significantly less than the controls, showing that DFP produced an acute supersensitivity to acetylcholine. At 24 hr the ED_{50} of acetylcholine was greater than the ED_{50} at 2 hr but still less than controls. The disappearance of supersensitivity and the return of normal sensitivity to acetylcholine was observed during days 4–10. Treatment with DFP for longer periods resulted in subsensitivity to acetylcholine as shown by the increase in ED_{50} on days 14–32.

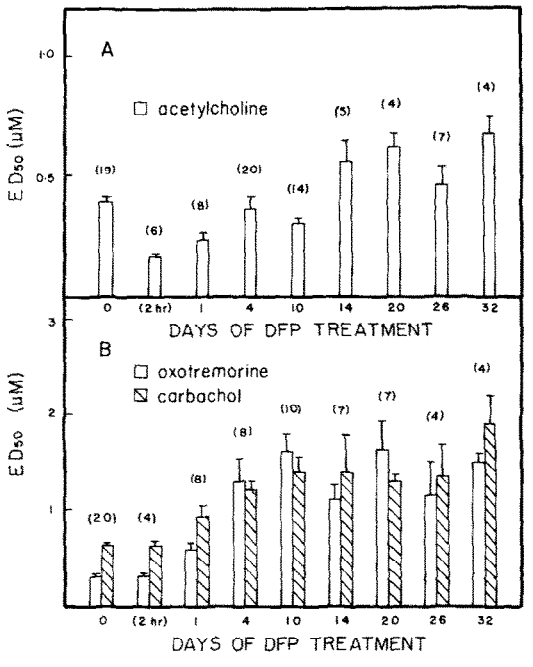

Fig. 1. Effects of DFP treatment on the sensitivity of isolated ilea to muscarinic agents. Panel A: ED_{50} values of ACh; panel B: ED_{50} values of oxotremorine and carbachol. Except for the ED_{50} values determined 2 hr after the first injection of DFP, all ED_{50}'s values were determined 24 hr after the previous injection of DFP as described in Methods. Mean values + S.E. are shown, and the number of rats in each group is indicated in parentheses.

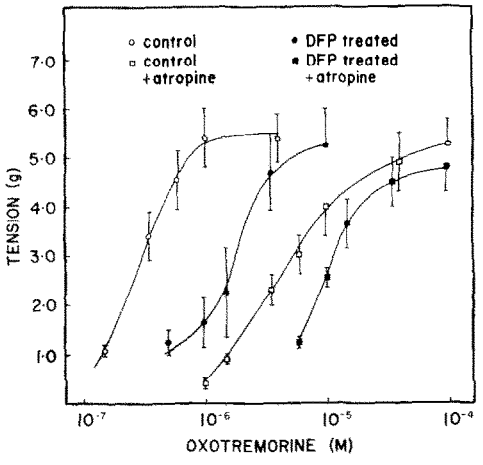

Fig. 2. Effects of DFP treatment on atropine antagonism of oxotremorine-induced contractions of the ileum. The dose–ratio of atropine was determined in ilea from control and 10-day DFP-treated rats as described in Methods. The concentration of atropine used in this experiment was 10^{-8} M. Mean values for tension ± S.E. are shown.

In contrast, the ilea of EFP-treated rats did not show supersensitivity to oxotremorine or carbachol. Figure 1B shows that the ED_{50} value of oxotremorine and carbachol were similar to controls at 2 hr and were increased moderately at 24 hr. The subsensitivity to oxotremorine or carbachol with the present DFP regimen attained a maximum on day 4. A comparison of ED_{50} value shows no significant differences in the sensitivity of the ileum to oxotremorine on days 4–32. Similar results were obtained with carbachol.

Atropine antagonism of oxotremorine-induced contraction of the ileum was determined in control and 10-day DFP-treated rats (Fig. 2) as described in Methods. At a concentration of 10^{-8} M, atropine caused a greater shift in the oxotremorine dose–response curve of control rats than of DFP-treated rats, as indicated by a decrease in the atropine dose ratio from a control value of 14 ± 1.2 to a value of 6.9 ± 1.6 in DFP-treated rats. The reduction in the atropine dose ratio suggests that DFP treatment decreased the affinity of muscarinic receptors in the ileum.

The effect of DFP treatment (10–14 days) on the specific binding of [^{3}H]QNB to the longitudinal muscle of the ileum is shown in Fig. 3. Specific [^{3}H]QNB binding was measured at six different concentrations of [^{3}H]QNB, ranging from 0.1 to 3.2 nM, in ilea from control and DFP-treated rats. [^{3}H]QNB binding obeyed mass action kinetics for a single class of independent receptors as indicated by the presence of linearity of Scatchard analysis of the binding data. Chronic DFP treatment (10–14 days) caused a significant reduction ($P < 0.05$) in the mean specific binding values determined at concentrations of 0.2, 0.4, 0.8 and 1.6 nM [^{3}H]QNB. This reduction in [^{3}H]QNB binding was primarily due to a decrease in the affinity of muscarinic receptors in the ileum. The mean K_D for [^{3}H]QNB was 0.43 ± 0.06 nM in DFP-treated rats and 0.25 ± 0.02 nM in controls ($P < 0.03$). In addition, the Scatchard plot shows that the

Table 1. Effects of DFP treatment on the displacement of ileal [^{3}H]QNB binding by various nonlabeled cholinergic drugs*

	Control		DFP-treated	
	K_i (μM)	N†	K_i (μM)	N†
Acetylcholine	0.95 (0.063–1.16)	4	2.0 (1.7–2.4)	4
Carbachol	2.0 (1.6–2.5)	6	5.5‡ (4.1–7.2)	6
Oxotremorine	0.093 (0.077–0.12)	9	0.33‡ (0.26–0.42)	9
Atropine	0.0055 (0.0037–0.008)	5	0.012 (0.008–0.017)	4

* Ninety-five per cent confidence intervals are indicated in parentheses beneath each K_i value.

† Number of experiments done on individual rats.

‡ Significantly different from control, $P < 0.05$.

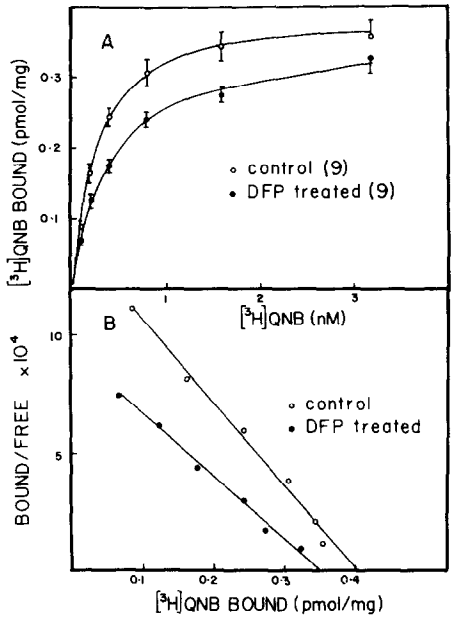

Fig. 3. Effects of DFP treatment on the specific binding of [^{3}H]QNB to the longitudinal muscle of the ileum. Panel A: [^{3}H]QNB binding was measured at various concentrations in control (○), and 10 to 14-day DEP-treated (●) rats. Mean binding values ± S.E. are shown, and the number of rats in each group is indicated in parentheses. The theoretical curve represents the least squares fit determined by nonlinear regression analysis. Panel B: Scatchard analysis of the mean binding values shown in panel A.

mean number of receptors in the ilea of DFP-treated rats was less than in the controls although the difference was not statistically significant.

Additional measurements of specific [^{3}H]QNB binding were done on ileal homogenates of thirty control and thirty DFP-treated rats to confirm the decrease in [^{3}H]QNB binding shown in Fig. 3. [^{3}H]QNB binding was measured with 0.8 nM [^{3}H]QNB, a concentration at which binding is near-maximal and the ratio of specific to nonspecific binding is high (10 : 1). DFP treatment produced a small but highly significant decrease in [^{3}H]QNB binding from 0.29 ± 0.01 pmoles/mg protein in the controls to 0.25 ± 0.008 pmole/mg protein in the 10 to 14-day DFP-treated rats ($P < 0.003$). Since no decreases of [^{3}H]QNB binding were measured when longitudinal muscle homogenates of control rats were incubated with 10^{-7} and 10^{-5} M DFP at 25° for 45 min, it is unlikely that the reduced [^{3}H]QNB binding observed in DFP-treated rats was due to a direct effect of DFP on muscarinic receptors. DFP at 10^{-7} and 10^{-5} M produced 80 and 100 per cent inhibition of ChE, respectively.

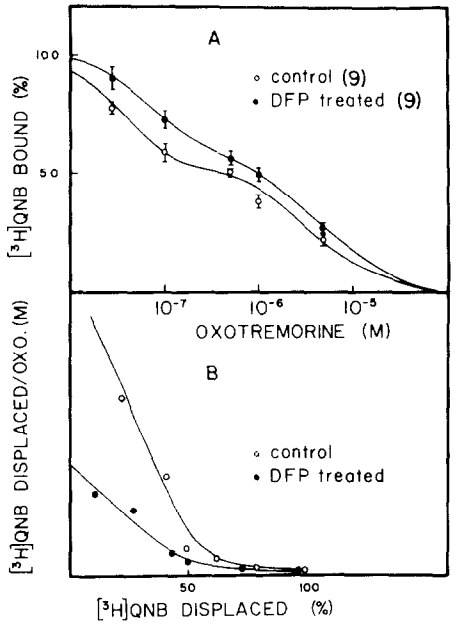

Fig. 4. Effects of DFP treatment on oxotremorine displacement of [^{3}H]QNB binding in the longitudinal muscle of the ileum. Panel A: [^{3}H]QNB binding was measured in the presence of various concentrations of oxotremorine in longitudinal muscle homogenates from control (○), and 10 to 14-day DFP-treated (●) rats. Mean binding values ± S.E. are shown, and the number of rats in each group is indicated in parentheses. The theoretical curve represents the least squares fit determined by nonlinear regression analysis. Panel B: Scatchard analysis of the mean binding values shown in panel A.

Table 2. Effects of DFP treatment on the ileal binding parameters of agonists*

Agonists	Treatment	N[b]†	K_H (μM)	N_H (%)	K_L (μM)	N_L (%)	K_L/K_H
Acetylcholine	Control	4	0.052 (0.035–0.072)	41 (36–46)	5.0 (3.8–6.5)	59 (54–64)	96
	DFP	4	0.15‡ (0.10–0.22)	42 (38–46)	8.0 (4.9–12)	58 (54–62)	53‡
Oxotremorine	Control	9	0.0086 (0.0060–0.012)	50 (45–55)	1.0 (0.8–1.3)	50 (45–55)	116
	DFP	9	0.0293‡ (0.020–0.043)	43 (38–48)	1.8 (1.3–2.5)	57 (52–62)	60‡
Carbachol	Control	6	0.038 (0.026–0.052)	42 (36–48)	12 (9.2–17)	58 (52–64)	313
	DFP	6	0.0757 (0.054–0.11)	37 (32–42)	20 (12–32)	63 (58–68)	262

* Ninety-five per cent confidence intervals are indicated in parentheses beneath each parameter estimate.

† Number of experiments done on individual rats.

‡ Significantly different from control, $P < 0.05$.

After 10–14 days of DFP treatment, an increase in the K_i of various nonlabeled cholinergic ligands was observed (Table 1), reflecting a decreased affinity of the muscarinic receptor for these drugs. Atropine displacement of [^{3}H]QNB binding was consistent with the law of mass action for a single binding site since Hill plots of the atropine/[^{3}H]QNB competition curves had slopes not significantly different from 1. Further analysis of the displacement of [^{3}H]QNB binding by oxotremorine in the ilea of control and 10 to 14-day DFP-treated rats is shown in Fig. 4. Scatchard plots revealed the presence of both high and low affinity agonist binding sites and that the concentrations of the high (N_H) and low (N_L) affinity sites are approximately equal. To determine the binding parameters of oxotremorine for the two binding sites, the competitive inhibition data were fitted to a two site binding equation by nonlinear regression analysis. Following DFP treatment, the dissociation constants of the high (K_H) and low (K_L) affinity sites increased from 0.0086 and 1.0 μM in controls to 0.0293 and 1.8 μM in DFP-treated rats, indicating that the affinities of both agonist binding sites had decreased. The increase in K_L was less than that of K_H, such that the ratio K_L/K_H decreased from 116 ± 19 in controls to 60 ± 10 in DEP-treated rats ($P < 0.01$). This decrease in K_L/K_H causes a steepening of the binding isotherm for oxotremorine and decreases the concavity of the Scatchard plot. Such a change would also increase the Hill coefficient for oxotremorine. The relative concentrations of the two agonist binding sites (N_L/N_H) did not change significantly following DFP treatment. Similar results presented in Table 2 were obtained for acetylcholine and carbachol.

DISCUSSION

The results reported here are in general agreement with our recent observations [11] concerning the effects of chronic DFP treatment on the binding of cholinergic ligands to rat striatal muscarinic receptors. The reduction in [^{3}H]QNB binding observed in both studies supports the proposal that the acquired tolerance to chronic ChE inhibition by organophosphorus compounds involves adaptive changes of cholinergic receptors [8–10].

The results of our isolated ileum experiments are consistent with the findings of previous investigators [3, 5, 17] and illustrate how ChE activity and tissue sensitivity affect the ED_{50} of acetylcholine for producing contractions. The initial supersensitivity to acetylcholine observed 2 hr after DFP can be attributed to potentiation of the effects of acetylcholine by ChE inhibition. This conclusion has been expressed by others [18] and is consistent with the observation that ileal sensitivity to the ChE resistant oxotremorine and carbachol was normal 2 hr after the first dose of DFP. During days 1–4 of DFP treatment, the loss of supersensitivity to acetylcholine, as shown by the increase in the ED_{50} of acetylcholine to normal values, paralleled the decrease in sensitivity to oxotremorine and carbachol, which attained a maximum on day 4 of DFP treatment. The normal sensitivity to acetylcholine on days 4–10 of DFP treatment most likely represents a balance between the potentiating effects of ChE inhibition and postjunctional subsensitivity. There is no adequate explanation, however, for the additional decrease in the sensitivity of the ileum to acetylcholine, observed after 10 days of DFP treatment, without concomitant decreases in sensitivity to oxotremorine and carbachol.

Our binding experiments showed that the antagonists [^{3}H]QNB and atropine obeyed the law of mass action for a single class of receptors. In contrast, agonist displacement of [^{3}H]QNB binding deviated from mass action kinetics as indicated by the flat binding isotherms which had Hill coefficients of less than 1. This observation has been reported in several studies [19–21]. To account for the complex nature of agonist binding, Birdsall and Hulme [22] have proposed the existence of high and low affinity agonist sites which have equal affinity for antagonists. The recent findings of Birdsall *et al.* [23] and Aronstam *et al.* [24] and the present findings are consistent with this model.

With regard to the two agonist binding sites, chro-

nic DFP treatment caused a greater reduction in the affinity of the high affinity agonist site. Consequently, there was a reduction in the ratio K_L/K_H and a slight steepening of the oxotremorine binding isotherm. These results are similar to those of Young [25] who reported an increase in the Hill coefficient of carbachol inhibition of propylbenzilylcholine mustard binding to the guinea pig ileum following desensitization. The decrease in K_L/K_H may indicate that agonist efficacy was decreased by DFP treatment since a correlation between efficacy and the ratio of the two agonist affinity constants has been reported [23, 26].

Although the relationship between the ED_{50} for smooth muscle contraction and the binding parameters of muscarinic cholinergic agonists is not fully understood [22, 26, 27], our results strongly suggest that muscarinic receptor subsensitivity is at least partially responsible for tolerance to chronic ChE inhibition. This hypothesis is supported by the observation that the K_i values of oxotremorine and carbachol increased 3-fold with DFP treatment which correlated with a 2 to 5-fold increase in the ED_{50} values of these agonists for eliciting contraction. Similarly, the 2-fold increase in the K_i of the antagonist atropine correlated with a decrease in the dose-ratio of atropine. Because of the complexities of agonist binding, however, it is not certain whether the subsensitivity of the ileum of DFP-treated rats is primarily due to a change in one or both agonist binding sites.

The present finding that DFP treatment decreased the dose–ratio atropine in the ileum appears to conflict with the report of Perrine and McPhillips [28] that chronic ChE inhibition with disulfoton reduced the sensitivity of the rat atrium to carbachol without changing the pA_2 of atropine. Since potent antagonists such as atropine bind to accessory sites adjacent to the muscarinic receptor [29, 30], it is possible that agonist affinity can decrease without a change in antagonist affinity in the atrium of disulfoton-treated rats. In this regard, selective alteration by *N*-ethylmaleimide of agonist binding but not of antagonist binding to neural membranes has been reported by Aronstam *et al.* [24]. However, the increase in the K_D of [^{3}H]QNB and the K_i of atropine and the decrease of the atropine dose–ratio found in the present study show that the affinity of muscarinic receptors for both antagonists and agonists was altered by DFP treatment.

The subsensitivity to cholinomimetics occurring with DFP treatment does not appear to be attributable to a direct effect of DFP on muscarinic receptors since no inhibition of [^{3}H]QNB binding was detected when longitudinal muscle homogenates were incubated with 10^{-5} M DFP, a concentration which abolished ChE activity. Although it is possible that DFP treatment caused postjunctional subsensitivity by a mechanism independent of ChE inhibition, the results of several investigations suggest that organophosphate-induced subsensitivity in muscarinic cholinergic innervated tissues is due to ChE inhibition and the subsequent accumulation of acetylcholine. In this regard, Bito and Dawson [10] have demonstrated that DFP-induced subsensitivity of the iris is blocked by decentralization and hemicholinium treatment. Organophosphate-induced subsensitivity in the guinea pig ileum has been shown to be blocked by concomitant administration of a reversible ChE inhibitor [31, 32]. Presumably, reversible ChE inhibitors protect ChE from irreversible phosphorylation by temporarily binding to the enzyme while the organophosphate is inactivated and eliminated. Recently, we demonstrated that chronic DFP treatment causes a reduction in striatal muscarinic receptor density which was prevented by concomitant physostigmine treatment and by chronic atropine treatment [11]. Interestingly, organophosphates have been shown to produce neuropathies in skeletal muscle by a mechanism independent of ChE inhibition [33, 34]; however, the evidence cited above suggests that, in muscarinic cholinergic innervated tissues, organophosphate-induced subsensitivity is caused by ChE inhibition. Thus, the results of this study suggest that muscarinic receptor subsensitivity is part of the adaptation to the accumulation of acetylcholine at cholinergic receptor sites as a result of ChE inhibition. Although it is possible that other pre- and postjunctional changes may underlie DFP-induced subsensitivity [18], the present findings establish a role for muscarinic receptor subsensitivity in the development of tolerance to the effects of organophosphorus ChE inhibitors.

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