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# Altered [$^3$H]Quinuclidinyl Benzilate Binding in the Striatum of Rats following Chronic Cholinesterase Inhibition with Diisopropylfluorophosphate

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SUMMARY

EHLERT, F. J., N. KOKKA AND A. S. FAIRHURST. Altered [$^3$H]quinuclidinyl benzilate binding in the striatum of rats following chronic cholinesterase inhibition with diisopropylfluorophosphate. *Mol. Pharmacol.* 17: 24–30 (1980).

Chronic administration of diisopropylfluorophosphate to rats at a dose regimen producing 90% inhibition of striatal cholinesterase caused a significant decrease in the density of [$^3$H]quinuclidinyl benzilate binding sites in homogenates of the striatum. The concentration of binding sites decreased from 1.39 ± 0.06 in controls to 0.82 ± 0.02 pmol/mg protein in diisopropylfluorophosphate-treated rats, and the half-time for the loss of binding sites was approximately 1.6 days. This decrease in [$^3$H]quinuclidinyl benzilate binding was not due to a direct effect of diisopropylfluorophosphate on muscarinic receptors since no inhibition of binding was produced by high concentrations of diisopropylfluorophosphate when added *in vitro*. An increase in the $K_i$ for inhibition of [$^3$H]quinuclidinyl benzilate binding by various nonlabeled cholinergic ligands was observed in chronic diisopropylfluorophosphate-treated rats, indicating that the affinity of the muscarinic receptors in the striatum had decreased. Following diisopropylfluorophosphate treatment, muscarinic receptors displayed a greater decrease in affinity to muscarinic agonists than to antagonists. Scatchard analysis of oxotremorine inhibition of [$^3$H]quinuclidinyl benzilate binding showed both high ($K_H$ = 0.01 ± 0.002 μM) and low ($K_L$ = 0.86 ± 0.18 μM) affinity binding sites in relative densities of 26 and 74%, respectively. Chronic administration of diisopropylfluorophosphate produced an increase of $K_H$ to 0.037 ± 0.008 μM, indicating a decrease in affinity but had no significant effects on $K_L$ or on the relative densities of the two sites. Similar results were obtained for acetylcholine and pilocarpine. In contrast, Scatchard analysis of the atropine inhibition of [$^3$H]quinuclidinyl benzilate binding showed a single class of antagonist binding sites. These decreases in affinity and density of muscarinic receptors in the striatum following chronic cholinesterase inhibition demonstrate that the tolerance to diisopropylfluorophosphate is, in part, a receptor mediated phenomenon.

## INTRODUCTION

Several studies have shown that the symptoms of excessive cholinergic stimulation caused acutely by organophosphorus ChE[2] inhibitors gradually diminish with chronic administration of these agents (1–4). This acquired tolerance to ChE inhibitors has been shown to be associated with a subsensitivity to cholinomimetics (5–8), which has led to suggestions that decreased receptor affinity (5, 9) and receptor density (10) may be involved in this tolerance. A reduction in nicotinic receptor density in the rat diaphragm has been reported by Chang *et al.* (1) following intermittent inhibition of ChE produced by daily administration of neostigmine. Their results thus suggest that tolerance to the peripheral nicotinic effects of anticholinesterase treatment is, in part, mediated via changes in receptors; however, it is not known whether such changes also occur in the muscarinic receptors in the brain. The purpose of the present study therefore was to determine directly, using the specific muscarinic affinity label [$^3$H]QNB, whether rat brain muscarinic receptors are altered and thereby possibly account for the subsensitivity to cholinomimetics which occurs after chronic ChE inhibition produced by DFP. The striatum

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[2] The abbreviations used are: ChE, cholinesterase; [$^3$H]QNB, 3-[$^2$H]quinuclidinyl benzilate; DFP, diisopropylfluorophosphate.



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was utilized in this study since this area is richest in muscarinic receptors (12).

## METHODS

*DFP treatment.* Male albino Sprague–Dawley rats weighing 200–250 g were utilized in these experiments. The animals were housed two or three per 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. DFP, 2 mg/kg was administered initially, followed by maintenance doses of 1 mg/kg DFP on alternate days after the second day. At various times DFP-treated rats were decapitated and the striatum was removed for the biochemical studies described below. Unless stated otherwise, rats were sacrificed 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 effect on striatal ChE.

Previous reports have shown that prior administration of a reversible ChE inhibitor protects ChE from phosphorylation by irreversible ChE inhibitors (13), so that in some experiments physostigmine (0.5 mg/kg, sc) was administered concurrently with DFP, with subsequent measurements of striatal ChE and [$^3$H]QNB binding activities. Also, in some experiments atropine (10 mg/kg, sc) was administered every 5 hr in addition to the usual dose of DFP, to determine the effects of chronic atropine treatment on [$^3$H]QNB binding in DFP-treated rats.

*Cholinesterase assays.* For the determination of ChE, the striatum was homogenized in a Potter–Elvehjem homogenizer in ice-cold 0.1 M $Na^+$-$K^+$ phosphate buffer of pH 8.0, at a concentration of 20 mg wet wt/ml buffer. Homogenates were kept at 0° and ChE activities measured within 4 hr of sacrifice. The ChE assay of Ellman *et al.* (14) was employed, with activities expressed as μmol acetylthiocholine hydrolyzed $min^{-1}$ mg $protein^{-1}$. Protein was determined by the method of Lowry *et al.* (15) using bovine serum albumin as standard.

*Binding assays.* Specific [$^3$H]QNB binding was determined after 1 hr at 25° and pH 7.4 according to the method of Yamamura and Snyder (16), with minor modifications. [$^3$H]QNB of specific activity 16 Ci/mmol was used, and was checked for radiochemical purity using two ethanol– and butanol–acetic acid–water systems. The striatum was first homogenized in a Potter–Elvehjem homogenizer, followed by a 10-sec homogenization with a Polytron at a setting of 5. $Na^+$-$K^+$ phosphate buffer, 0.05 M and pH 7.4, was used, the homogenate concentration being 10 mg wet wt/ml. Triplicate tubes contained 100 μl of homogenate in a final volume of 2 ml, with parallel systems containing in addition $10^{-6}$ M atropine. Specific [$^3$H]QNB binding was calculated as the total binding minus that occurring in presence of atropine; all binding data in the text refer to specific binding. For measurement of the competitive displacement of [$^3$H]QNB binding, a final concentration of 0.8 nM [$^3$H]QNB was used. At this concentration less than 5% of the total [$^3$H]QNB was bound. When acetylcholine inhibition of [$^3$H]QNB binding was studied, the assays contained $10^{-6}$ M physostigmine to prevent enzymatic hydrolysis of acetylcholine.

In experiments in which both atropine and DFP were administered *in vivo*, striatal homogenates were washed three times to ensure that no atropine was present during the binding assay. Washing was accomplished by centrifugation of the striatal homogenate at 37,000 *g* for 10 min followed by resuspension of the pellet in fresh 50 mM phosphate buffer. Incubations (10, 20, and 40 min) at 37° in a Dubnoff shaking incubator preceded the first, second, and third washes, respectively, to allow dissociation of receptor-bound atropine. The results of these experiments were compared with those in which striatal homogenates from control and DFP-treated rats without atropine were washed according to the same procedure.

*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

$$B = \sum_{j=1}^{n} \frac{XN_j}{X + K_j}, \qquad [1]$$

where $B$ is the concentration of bound [$^3$H]QNB, $X$ is the concentration of free [$^3$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. In measurements of the displacement of [$^3$H]QNB binding by nonlabeled ligands, $B$ is the percentage [$^3$H]QNB displayed by a given concentration $X$ of nonlabeled drug and $K$ is the apparent dissociation constant of the nonlabeled drug.

An iterative computer program written in Basic on a PDP 11/40 computer was used to determine the least-squares fit to Eq. [1]. The program described by Feldman (17), which derives the least squares to fit to the Scatchard plot, was modified to determine the least squares fit to the binding isotherm. These modifications included the formation of (a) a matrix containing the differences between observed and predicted binding values, and (b) a two-dimensional matrix containing the partial derivatives of the Langmuir relationship with respect to each parameter solved at each drug concentration. These matrices replaced those labeled $H$ and $M$, respectively, in the procedure outlined by Feldman (17).

The apparent dissociation constants of nonlabeled ligands determined in displacement of [$^3$H]QNB binding studies are overestimations of the true dissociation constants. The true dissociation constants were calculated as

$$K_{(true)} = K_{(App)}/1 + \frac{\{[^3H]QNB\}}{K_{QNB}}, \qquad [2]$$

where $K_{(true)}$ is the true dissociation constant of the nonlabeled ligand, $K_{(App)}$ is the apparent dissociation constant derived from the least-squares fit to equation [1], {[$^3$H]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. $K_i$ values for nonlabeled ligands were calculated in a similar manner

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

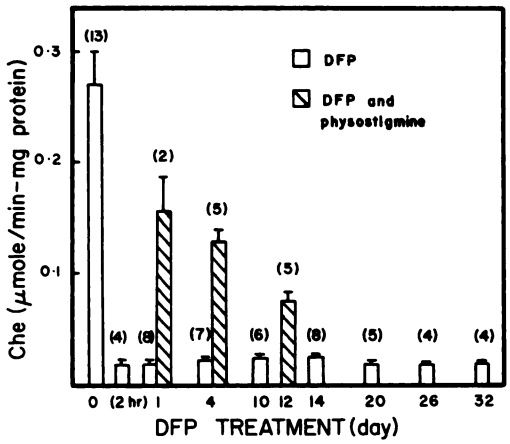


FIG. 1. *Effects of DFP treatment and concomitant administration of DFP and physostigmine on striatal ChE activity*

Mean values ± SE are shown, and the number of measurements, each made on an individual rat, is indicated in parentheses.

where ($IC_{50}$) is the concentration of nonlabeled ligand which caused half-maximal displacement of [$^3$H]QNB binding.

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

*Drugs and chemicals.* [$^3$H]QNB was obtained from Amersham Corporation; DFP from Aldrich Chemical Company; acetylcholine, acetylthiocholine, atropine, dithiobisnitrobenzoic acid, physostigmine, and pilocarpine from Sigma Chemical Company; oxotremorine was kindly provided by Dr. Donald Jenden.

## RESULTS

The initial injection of 2 mg/kg DFP produced signs of excessive cholinergic stimulation characterized by diarrhea, tremor, salivation, and lacrimation. Similar effects of reduced intensity were produced by the maintenance dose of 1 mg/kg on Days 3 and 5 but little or no signs of cholinergic stimulation were observed on Days 7–9, indicating that tolerance to DFP had developed.

The dose regimen of DFP used in this study produced 90% inhibition of ChE as measured 24 hr after the last injection of DFP (Fig. 1). This inhibition of ChE was partially blocked by concomitant administration of the reversible ChE inhibitor physostigmine, 0.5 mg/kg (Fig. 1). ChE inhibition in the striatum of DFP-treated rats was reduced by physostigmine from 90% to 48, 57, and 75% on Days 1, 4, and 12, respectively. The results show that physostigmine protected ChE from DFP inactivation and that a successive lowering of ChE activity occurred with chronic administration of physostigmine–DFP.

The half-time for recovery of ChE in the striatum and other brain regions following a single dose of DFP (2 mg/kg) is shown in Fig. 2. This relatively long half-time of recovery suggests that ChE activity in the striatum did not fluctuate considerably during the 48-hr intervals between the maintenance dosage injections of DFP.

The effect of DFP treatment (10–14 days) on the

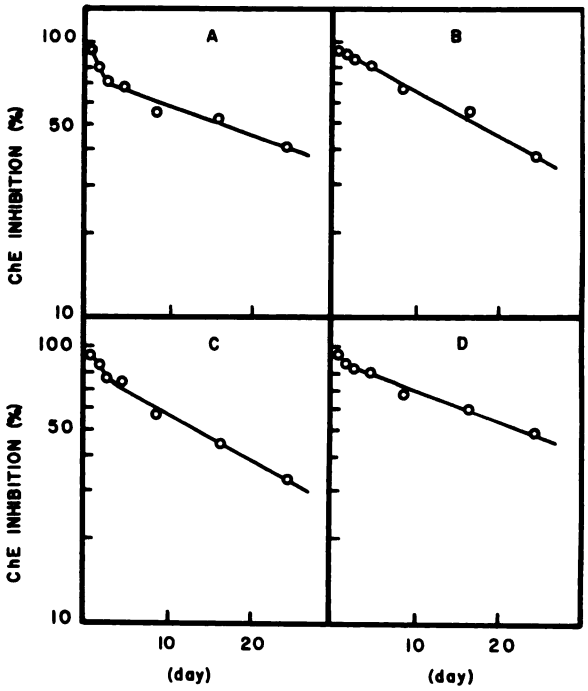


FIG. 2. *Recovery of brain ChE activity following DFP administration*

(A) hypothalamus; (B) striatum; (C) midbrain–thalamus; (D) cortex. A single 2 mg/kg dose of DFP was administered and ChE activity was subsequently determined at various times. Each point represents the mean of four separate determinations.

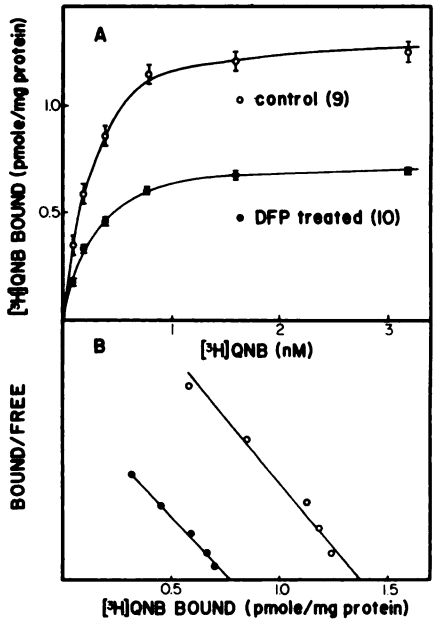


FIG. 3. *Effects of DFP treatment on the specific binding of [$^3$H]QNB to the striatum*

(A) [$^3$H]QNB binding was measured in various concentrations in control, ○, and 10- to 14-day DFP-treated, ●, rats. Mean values ± SE are shown, and the number of experiments, each done on individual rats, is indicated in parentheses. (B) Scatchard analysis of the mean binding values shown in (A).

binding of [$^3$H]QNB to striatal homogenates is shown in Fig. 3A. [$^3$H]QNB binding followed the law of mass action for a single class of independent receptors which resulted

in linear Scatchard plots (Fig. 3B). Specific [$^3$H]QNB binding was significantly reduced at all concentrations measured in striatal homogenates from rats treated with DFP. This reduction in [$^3$H]QNB binding was due primarily to a decrease in the density of muscarinic receptors in the straitum, since the density of the [$^3$H]QNB binding site decreased from 1.39 ± 0.06 pmol/mg protein in controls to 0.82 ± 0.02 pmol/mg protein after DFP treatment. A nonsignificant increase in the dissociation constant (i.e., decrease in affinity) of [$^3$H]QNB was observed; namely, the $K_D$ for QNB increased from 0.24 to 0.03 nM in controls to 0.32 ± 0.03 nM after DFP treatment.

The rate at which [$^3$H]QNB binding decreased was estimated by measuring binding at various times during DFP treatment. Figure 4 shows that binding was significantly decreased at all times except after 2 hr following the first injection of DFP. A near maximum decrease in binding was seen after 4 days of DFP treatment, since comparison of binding after 4, 10, 14, and 30 days of DFP showed no significant differences. The rate of decrease

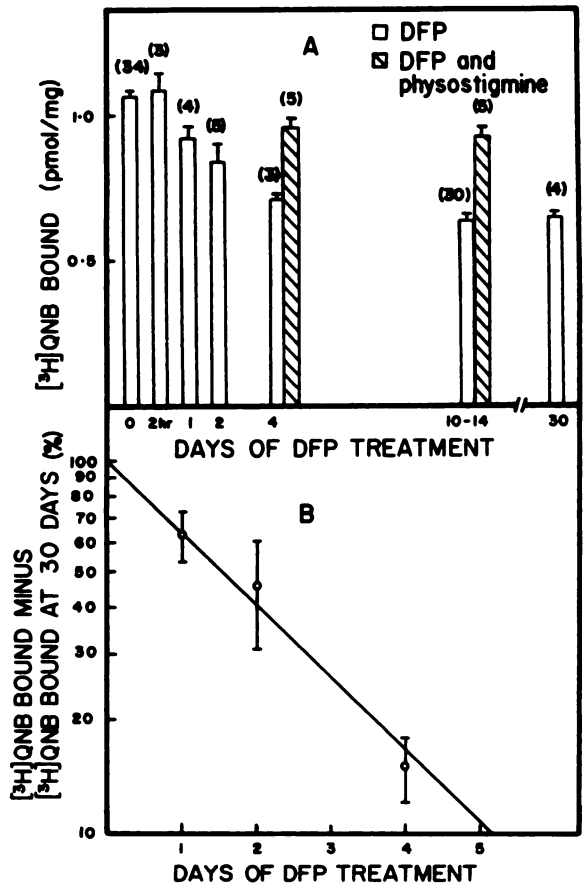


FIG. 4. *Time course for reduced striatal [$^3$H]QNB binding during DFP treatment*

(A) Striatal [$^3$H]QNB binding was measured at various times in rats treated with DFP. The effects of concomitant administration of physostigmine and DFP on [$^3$H]QNB binding are shown also. Mean values ± SE are shown, and the number of experiments, each done on individual rats, is indicated in parentheses. (B) Rate of loss of [$^3$H]QNB binding sites during chronic DFP treatment. The differences ± SE between the mean value on a given day and the mean binding on the 30th day of DFP treatment are shown. The data were calculated from the values shown in (A).

TABLE 1

*Effects of chronic atropine treatment on DFP-induced changes in striatal [$^3$H]QNB binding*

| Treatment | [$^3$H]QNB bound |
|---|---|
| | *(% control)* |
| Control | 100 ± 10 |
| DFP | 62 ± 7* |
| DFP + atropine | 96 ± 5** |

* $p < 0.02$, from control.
** $p < 0.01$, from DFP treated.

TABLE 2

*Effects of DFP treatment on the displacement of striatal [$^3$H]QNB binding by various nonlabeled drugs*

| | Control $K_i$ (μM) | | DFP treated $K_i$ (μM) | |
|---|---|---|---|---|
| Acetylcholine | 6.5 | (6)[a] | 10.7 | (6) |
| Oxotremorine | 0.38 | (6) | 0.76* | (8) |
| Pilocarpine | 3.5 | (5) | 5.1* | (6) |
| Atropine | 0.00025 | (6) | 0.00032 | (6) |

[a] ( ) Number of rats.
* $p < 0.05$.

in [$^3$H]QNB binding approximated a first order process, with a half-time of 1.6 days. No decreases of [$^3$H]QNB binding were detected when striatal homogenates of control rats were incubated with $10^{-7}$ and $10^{-5}$ M DFP, although these concentrations of DFP produced 80 and 100% inhibition of ChE, respectively. Since the degree of ChE inhibition (100%) produced by $10^{-5}$ M DFP *in vitro* was greater than that produced by chronic DFP treatment, and since no inhibition of [$^3$H]QNB binding by $10^{-5}$ M DFP added *in vitro* was detected, it is unlikely that DFP when administered *in vivo* produced the reduction in [$^3$H]QNB binding by directly binding to striatal muscarinic receptors.

Concomitant administration of physostigmine partially blocked the decrease in [$^3$H]QNB binding caused by DFP treatment (Fig. 4). In rats receiving both DFP and physostigmine, decreases of 10% in [$^3$H]QNB binding were detected on Days 4 and 14 of DFP treatment whereas decreases of 35% were detected on the same days in rats receiving only DFP.

Chronic atropine treatment (10 mg/kg every 5 hr) also blocked the decrease in striatal [$^3$H]QNB binding caused by chronic DFP treatment (Table 1). In rats receiving both DFP and atropine, a 5% decrease in [$^3$H]QNB binding was detected in the striatum on the fourth day of drug treatment in contrast to a 39% decrease in the striatum of rats receiving DFP only. The binding data presented in Table 1 were obtained on washed striatal homogenates, as described in METHODS.

After 10–14 days of DFP treatment an increase in the $K_i$ of various nonlabeled cholinergic agents was observed (Table 2), reflecting a decreased affinity of the muscarinic receptor for these drugs. Cholinergic agonists displayed greater relative changes after DFP treatment than did the antagonist atropine. Further analysis of the displacement of [$^3$H]QNB binding by oxotremorine in the striatum of control and 10- to 14-day DFP-treated animals is shown in Fig. 5. Scatchard plots revealed the presence of

both high and low affinity agonist binding sites, and that the concentration of high affinity sites ($N_H$) was less than the concentration of low affinity sites ($N_L$). Following DFP treatment the dissociation constants of the high ($K_H$) and low ($K_L$) affinity sites increased from 0.01 ± 0.002 μM and 0.86 ± 0.18 μM, respectively, in controls to 0.037 ± 0.008 μM and 1.08 ± 0.15 μM in DFP-treated animals, indicating that the affinities of both sides had decreased. However, the relative increase in $K_H$ was greater than that of $K_L$, such that the ratio of $K_L/K_H$ decreased from 92 ± 18 in controls to 40 ± 10 in DFP rats ($p < 0.05$). This decrease in $K_L/K_H$ caused a steepening of the binding isotherm for oxotremorine and hence an increase in the Hill coefficient. The relative densities of the two agonist binding sites did not change significantly following DFP treatment. Similar results were found with acetylcholine and pilocarpine after DFP treatment and are shown in Table 3.

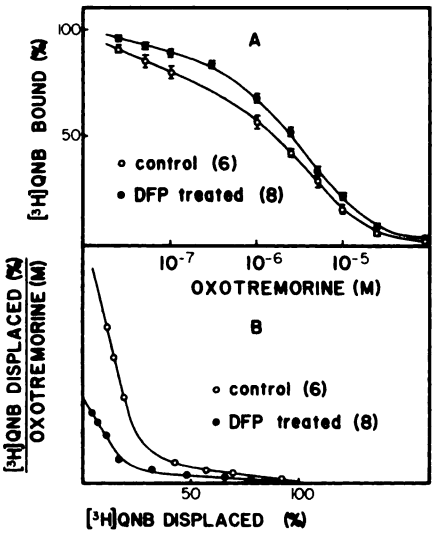


FIG. 5. *Effects of DFP treatment on oxotremorine displacement of [³H]QNB binding in the striatum*

(A) [³H]QNB binding was measured in the presence of various concentrations of oxotremorine in striatal homogenates from control, ○, and 10- to 14-day DFP-treated, ●, rats. Mean binding values ± SE are shown, and the number of experiments, each done on individual rats, is indicated in parentheses. (B) Scatchard analysis of the mean binding values shown in (A).

## DISCUSSION

The results presented here demonstrate that the tolerance acquired to chronic ChE inhibition is, at least in part, a receptor-mediated phenomenon. The predominant effect of DFP treatment on [³H]QNB binding was to reduce the number of [³H]QNB binding sites. This decrease in [³H]QNB binding was not due to a direct effect of DFP on muscarinic receptors since no inhibition of [³H]QNB binding was detected following incubation of striatal homogenates with $10^{-5}$ M DFP, a concentration that completely abolished ChE activity. In addition, no decrease in [³H]QNB binding was detected 2 hr after the systemic injection of 2 mg/kg DFP when ChE activity in the striatum was inhibited 90%.

These results suggest that DFP-induced alterations in striatal [³H]QNB binding are caused by ChE inhibition and the resulting accumulation of ACh at cholinergic receptor sites. This hypothesis is reinforced by the observation that DFP-induced alterations of [³H]QNB binding were blocked by concomitant administration of physostigmine, a reversible ChE inhibitor that protects ChE from irreversible phosphorylation by DFP. Thus it is unlikely that DFP produced a decrease in [³H]QNB binding by interacting with postsynaptic elements other than ChE. The present finding that chronic atropine administration blocked DFP-induced decreases of striatal [³H]QNB binding is also consistent with the view that accumulation of acetylcholine at receptor sites is necessary for the production of a muscarinic receptor deficit during DFP treatment.

The time course for the reduction of [³H]QNB binding in striatum during DFP treatment approximated a first order process with a half-time of about 1.6 days. If this

TABLE 3

*Effects of DFP treatment on the striatal binding parameters of agonists*[a]

| | $n$[b] | $K_H$ (μM) | $n_H$ (%) | $K_L$ (μM) | $n_L$ (%) | $K_L/K_H$ |
|---|---|---|---|---|---|---|
| Acetylcholine | | | | | | |
| Control | (6) | 0.048 (0.029–0.079) | 24 (18–30) | 12.5 (11.5–13.4) | 76 (70–82) | 259 |
| DFP treated | (6) | 0.166[c] (0.127–0.218) | 27 (21–33) | 25.6[c] (23.7–27.7) | 73 (67–79) | 154 |
| Oxotremorine | | | | | | |
| Control | (6) | 0.01 (0.007–0.015) | 26 (20–32) | 0.86 (0.55–1.34) | 74 (68–80) | 93 |
| DFP treated | (8) | 0.037[c] (0.023–0.059) | 22 (18–26) | 1.08 (0.81–1.43) | 78 (74–82) | 40[c] |
| Pilocarpine | | | | | | |
| Control | (5) | 0.15 (0.067–0.34) | 14 (8–20) | 4.94 (4.61–5.29) | 86 (80–92) | 34 |
| DFP treated | (6) | 0.49 (0.24–1.00) | 19 (15–23) | 8.01[c] (7.62–8.42) | 81 (77–85) | 16[c] |

[a] 95% confidence intervals are indicated in parentheses beneath each parameter estimate.

[b] Number of experiments, each done on individual rats.

[c] Significantly different from control, $p < 0.05$.

reduction in the density of muscarinic receptors was due to a decrease synthesis or to enhanced receptor degradation, then values of 1.6 or 2.7 days, respectively, can be calculated for the half-time of muscarinic receptor turnover in the control rat striatum. These estimations are based on the assumptions that there is a first order rate of receptor degradation and that DFP treatment produced rapid changes in the rates of synthesis or degradation relative to the half-time for receptor turnover. However, the calculated half time for the loss of [$^3$H]-QNB binding sites might bear little relationship to receptor turnover because other compensatory mechanisms could have produced the decrease in [$^3$H]QNB binding.

The attenuation of such pharmacological effects of DFP as diarrhea, tremor, salivation, and lacrimation correlated with the time course for decreased [$^3$H]QNB binding in the striatum. Although these effects of DFP are the results of excessive cholinergic stimulation in many areas of the nervous system, it is likely that the attenuation was the result of decreased muscarinic receptor sensitivity produced by changes similar to those observed in the striatum.

Previously we reported a similar time course for the production of tolerance in the isolated ileum after treating rats with the same DFP regimen used in this study (18). Maximum resistance to the effects of carbachol and oxotremorine developed after 4 days of DFP treatment. This subsensitivity to cholinomimetics was associated with a decrease of [$^3$H]QNB binding binding to the longitudinal muscle of the ileum. Behavioral tolerance (19) and peripheral tolerance in the isolated ileum (20) have been shown by other investigators to develop at much slower rates (10–20 days) with chronic DFP treatment. However, in those studies DFP was administered under a less vigorous dose regimen of 1 mg/kg initially, followed by 0.5 mg/kg every third day. These results suggest that tolerance to DFP develops at a slower rate when cholinesterase is inhibited to a lesser extent.

The dissociation constant for [$^3$H]QNB and the $K_i$ for atropine, acetylcholine, oxotremorine, and pilocarpine in striatum of normal rats are in general agreement with the values reported by Yamamura and Snyder (21) and Gilbert *et al.* (22) for the rat brain. Following DFP treatment cholinergic agonists displayed a greater decrease in affinity than antagonists for the muscarinic receptor. Although muscarinic agonists and antagonists displace one another at the same binding site, the critical moieties involved in binding are different (23, 24) and the binding affinity of muscarinic antagonists may be augmented by interaction of additional sites adjacent to the receptor (24). It is possible that chronic DFP treatment produces mainly a change in the agonist binding region of the receptor. A deficit of binding in this region would preferentially decrease agonist affinity while producing lesser effects on antagonist affinity.

The displacement of [$^3$H]QNB binding by atropine followed the law of mass action for a single class of receptors and, therefore, the calculated $K_i$ for atropine is equivalent to its dissociation constant. In contrast, the displacement of [$^3$H]QNB binding by the agonists acetylcholine, oxotremorine, and pilocarpine deviated from single site mass action kinetics. Binding isotherms of the agonist displacement of [$^3$H]QNB binding data had shallow slopes with Hill coefficients less than one. The most likely explanation is that agonists bind to two classes of muscarinic receptors that have equal affinity for antagonists (25–27). The high and low affinity dissociation constants for acetylcholine and oxotremorine determined from striatal homogenates in this study agree generally with the reciprocals of the affinity constants reported by Birdsall *et al.* (26). Also, the correlation between agonist efficacy and the ratio of high to low affinity constants ($K_L/K_H$ in this study) for agonists observed by Birdsall is confirmed by the present finding that the agonists acetylcholine and oxotremorine had $K_L/K_H$ ratios of 259 and 93, respectively, while the partial agonist pilocarpine had a lower ratio of 34.

Although the meaning of the decrease in $K_L/K_H$ cannot be firmly established at the present time, the correlation between agonist efficacy and the magnitude of $K_L/K_H$ suggests that agonist efficacy in the striatum decreased following chronic DFP treatment. Since a decrease of $K_L/K_H$ has the effect of increasing the Hill coefficient for agonists, present results are similar to those of Young (28) who found an increase in both the $IC_{50}$ and Hill coefficient for carbachol inhibition of [$^3$H]propylbenzylcholine mustard binding in the desensitized guinea pig ileum.

Our results suggest that adaptive mechanisms exist that produce a decrease in both the density and affinity of muscarinic receptors after prolonged elevation of endogenous levels of ACh. Although decreased muscarinic receptor binding was measured after chronic 90% inhibition of cholinesterase, it seems possible that in the normal animal such mechanisms mediating desensitization may be part of a feedback system that maintains the integrity of synaptic transmission.

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