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Effect on Striatal Dopamine Metabolism and Differential Motor Behavioral Tolerance Following Chronic Cholinesterase Inhibition with Diisopropylfluorophosphate

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FERNANDO, J. C R., B. HOSKINS AND I. K. HO. *Effect on striatal dopamine metabolism and differential motor behavioral tolerance following chronic cholinesterase inhibition with diisopropylfluorophosphate* PHARMACOL BIOCHEM BEHAV **20**(6) 951–957, 1984 —Rats were treated with diisopropylfluorophosphate (DFP), using 1 or 2 mg/kg acutely, or with 1 mg/kg daily for 4, 14 or 28 days. Their behaviors and striatal dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) levels were studied The behaviors tremors, chewing-movements and hind-limb abduction induced by DFP increased in a steeply dose-dependent manner. Chronic treatment for up to 28 days produced biphasic patterns of change for all the behavioral parameters Tremor occurred in a complex spectrum of slow to intense fast types Except for chewing, tolerance developed for these parameters, but at different rates. After acute treatment striatal DA and DOPAC levels were altered and the DOPAC/DA ratios were consistently increased within about the first two hr, suggesting an increased turnover of DA. After chronic treatment for 4 and 14 but not 28 days, both DA and DOPAC levels were decreased without a change in their ratios. It is suggested that the changes in DA metabolism arose secondarily to an elevation of brain acetylcholine following cholinesterase inhibition. A prolonged change in the levels or turnover of DA could be responsible for increase of postsynaptic DA receptor density previously found by us [36], which might then partly mediate the behavioral tolerance to DFP.

DFP Behavior Chewing movements Dopamine DOPAC Tremor Cholinesterase inhibitors

DIISOPROPYLFLUOROPHOSPHATE (DFP) is a potent and irreversible organophosphate acetylcholinesterase (AChE) inhibitor producing peripheral and central cholinergic over-stimulation. However, tolerance to these effects develops upon chronic exposure to the compound despite a high degree of cholinesterase inhibition, in animals [2] and also in humans [10]. Most of the previous work on this has been on the involvement of the cholinergic system itself in the mediation of behavioral tolerance to DFP [12, 24, 28, 31].

However, cholinergic mechanisms alone do not adequately explain this tolerance for the following reasons. The tolerance develops at markedly varying rates for different behavioral alterations, although they tend to have similar on-set times. Thus, decrements in food intake and shock avoidance caused by chronic DFP-treatment recovered well ahead of those in water intake and response inhibition [30], and a short tolerance time has been observed for the tremors [23,27]. DFP produces a particularly substantial reduction of AChE activity in the striatum [21,36], a brain area playing a major role in the regulation of motor function, the disruption of which leads to such effects as tremors. Cholinergic drugs have been shown to influence other transmitter pathways such as those innervating the striatum-basal ganglia system [17] which is rich in monoamines like dopamine (DA) [4] as well as in ACh [33], indicating the existence of cholinergic-dopaminergic interactions. Thus, cholinergic agonists and antagonists increase and decrease or prevent agonists from increasing, respectively, the turnover or metabolism of DA in the striatum [6, 7, 18, 20, 25, 38]. Also, it has been reported [15] that chronic exposure to organophosphates such as Mipafox markedly reduced striatal DA levels. Recently, we have shown that DA receptor density was increased in the rat striatum after chronic treatment with DFP [36].

We therefore investigated the involvement of the

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dopaminergic system further. The effects of acute and chronic administration of DFP on striatal levels of DA and its metabolite, dihydroxyphenylacetic acid (DOPAC), and development of tolerance to three distinctive DFP-induced motor behaviors (tremors, chewing-movements and hind-limb abduction), the latter two of which have not been characterized previously, were studied.

METHOD

Animals

Male Sprague-Dawley rats (Charles River Ltd., Wilmington, MA) with initial weights of 180–220 g were used. They were housed four to a cage with food and water ad lib in a temperature-controlled room (25±1°C) with 12-hr light/dark cycles.

DFP Treatment

Freshly prepared solutions of DFP (Calbiochem Lot No 103288) in saline were used as recently reported [23,36]. They were administered subcutaneously as 1 ml/kg into the flank, while an equivalent volume of saline was given to the control animals. Single doses of 1 or 2 mg/kg of DFP were used for acute treatment while a daily dose of 1 mg/kg was employed for chronic treatment. We have reported that using the same batch of DFP at a dose of 1 mg/kg SC, striatal AChE was inhibited by 50% (84% for 2 mg/kg) at 6 hr after and by 75% 4 or 14 days after chronic treatment [36].

Behavioral Analyses

Rats were individually isolated in observation cages These were transparent plastic rat cages each having a stainless steel grid resting off its floor. Each cage was divided into four compartments with three fitted opaque plastic sheets and was covered with a transparent plastic lid with several ventillation holes. A thin layer of bedding, but neither food nor water, was available; the grid prevented the animals from biting the bedding material or feces.

Observations were made during the hr immediately preceding (chronic treatment only) and 1 hr (0.5 hr also for acute treatment) periods after injections. The behaviors, chewing-movements (without biting or gnawing of objects), tremors and hind-limb abduction were separately scored on each rat, for a duration of 5 min repeated at 15 min intervals, starting at 15 min after treatment. The scoring scale utilized was: 0=none, 1=slight, 2=moderate and 3=severe, for the two behaviors other than tremor The latter was scored as 0=none, 1=distinct slow tremor of head, 2=fast tremor (high frequency vibrations) of head, trunk or limbs, 3=intense fast tremor; the scale was comparable to that described previously [37] for oxotremorine tremors. The scores for the intervals were summed and the maximum possible score for a 1 hr period for each behavior was 12 (6 for 0.5 hr) per rat. In general, eight animals per treatment group were used

Neurochemical Analyses

At selected times after treatment, the animals were killed by head-focused microwave irradiation (at 3 8 kw 2450 MHz for 2 sec; Metabostat, 4094, Thermex Inc.), usually between noon and 4:00 p.m. The brains were dissected on ice into unilateral striata and stored at −20°C until analyzed.

Both DA and DOPAC were assayed in the same striatal halves weighing approximately 40 mg. The extraction procedure was that used previously [14] except for minor modifications. The tissues were homogenized in 1 2 ml of n-butanol containing 0.01 M $HClO_4$ and centrifuged at 1,500×g for 10 min. One ml of each of the supernatants was shaken for 5 min with 2 ml n-heptane and 300 μl 0.1 N HCl containing 1 mg% ascorbic acid. Brief centrifugation separated the lower aqueous phase containing DA from the upper organic layer which containd DOPAC. Next, 2.6 ml of the organic phase were removed and shaken for 5 min with 300 μl of 0.5 M Na_2HPO_4/NaH_2PO_4 buffer, pH 7.4, containing 2 mg % ascorbic acid. Centrifugation separated the aqueous fraction now containing DOPAC. The residual organic layers were discarded by aspiration. The DOPAC fraction was immediately acidified with 30 μl 6 N HCl.

DA and DOPAC were assayed essentially according to the fluorometric methods of Laverty and Taylor [22] and Westerink and Korf [38], respectively

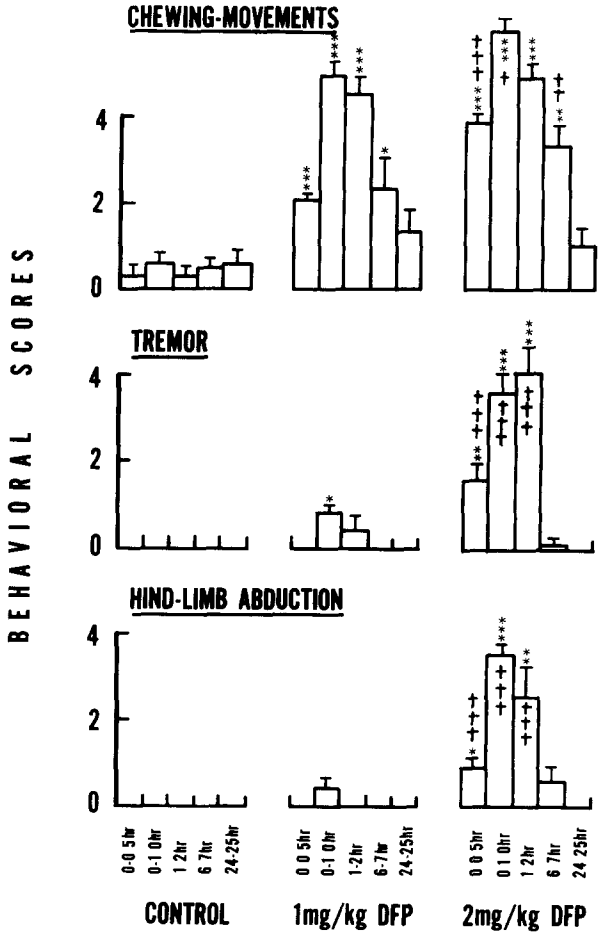

FIG 1 Effect of acute treatment with different doses of DFP on behaviors. Values are means±S E. of individual scores on 8 rats per group. Behaviors were rated during 0 5 or 1 0 hr periods and statistical differences were analyzed by Kruskal-Wallis multicomparison procedures (see the Method section) $p<0.05$, $p<0$ 01 and $p<0$ 001 compared with control (*,**,***) or 1 mg/kg dose (†,††,†††).

Statistical Analysis

Significance of differences within and across groups in behavioral experiments were analyzed by the Kruskal-Wallis

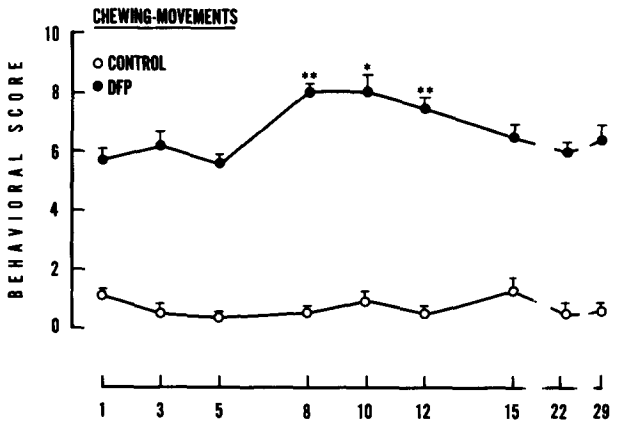

FIG. 2 Chewing-movements following chronic treatment with DFP (1 mg/kg daily) Values are means±S.E. of individual rat scores. Control, n=8, DFP, n=10 Behaviors were examined during the first hr of treatment. DFP values were compared with corresponding pre-injection and control values by the Mann-Whitney U-test $p<0.001$. Also, DFP 8th–12th values were compared with the 1st by Kruskal-Wallis procedure· *($p<0.05$) and **($p<0.01$)

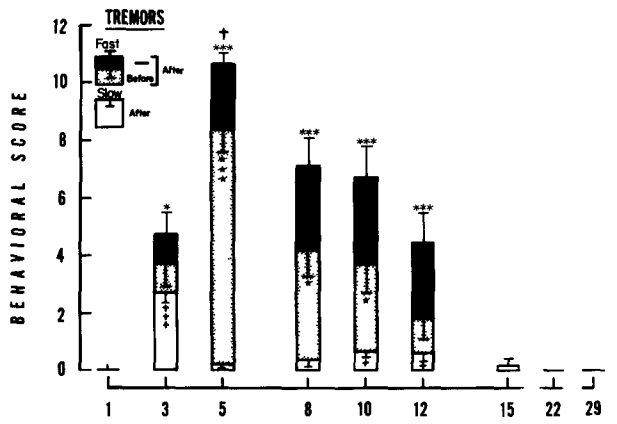

FIG. 3. Tremors following chronic treatment with DFP Values are means±S E Slow and fast tremor scores are separately given Mann-Whitney U-test was used for analysis of differences **†($p<0.05$) and ***†††($p<0.001$) compared with pre-injection (†) ★ ★★★

multiple pairs comparison procedures in the case of the acute-treatment data, and additionally by the Mann-Whitney U-test for the chronic data. Neurochemical data were analyzed by the Student's t-test.

RESULTS

Behavioral Effects of DFP-Treatment

Acute treatment· A prominent effect occurring within 15 min of DFP injections was the intermittent chewing-movements of the jaws and tongue without actually chewing. These were brief but frequent and were sometimes preceded by yawning. Interestingly, controls also displayed this behavior but only slightly and occasionally (Fig. 1). The chewing scores in the first half hr increased in a dose-dependent manner for 1 and 2 mg/kg and they were maximal during the first two hr. Chewing scores of DFP-treated rats were still significantly increased over those of controls at the seventh hr but were declining ($p<0.05$ compared with DFP-1 hr, for either dose). Although some chewing occurred during the 25th hr, the values were not significantly different from those of control animals.

Tremor (Fig. 1) was not observed in the control group of rats whereas slow tremor was clearly present in the 1 mg/kg DFP group during the second half of the first hr. After 2 mg/kg DFP, both slow and fast tremors occurred; the scores were significantly greater than after the lower dose even in the first half hr. By the seventh hr, tremor had greatly subsided so that the scores were not significantly different from control values (but $p<0.01$ compared with DFP-1 hr, for 2 mg/kg dose).

Hind-limb abduction (Fig. 1) also was displayed as dose-dependent increases. The hind-limb abduction scores were also maximal during the first two hr. After the seventh hr the behavior disappeared and the scores were not significantly different from control values ($p<0.05$ vs. DFP-1 hr, for 2 mg/kg).

Peripheral cholinergic effects such as salivation and defecation increased dose dependently.

Chronic treatment Only the 1 mg/kg dose of DFP could be used for daily treatment, since the repeated administration of higher doses was lethal. The behaviors were scored before and after every 2nd or 3rd treatment starting with the first.

The chewing scores after injections for the DFP-treated rats were significantly greater than pre-injection and control values throughout the 4 weeks study period (Fig. 2). They peaked at about the 8th to 12th treatment, the values being significantly higher than those after the first treatment. The pre-injection chewing scores for the DFP-group were not significantly different from the corresponding control values (not shown).

Scores for the slow and pre- and post-injection fast tremors are separately displayed in Fig. 3. Slow tremor was slight after the first two injections but showed a marked and highly significant rise after the 3rd treatment compared with pre-injection (0±0) and control scores. It was diminished after the later treatments (but was significantly higher than pre-injection and control values after the 10th and 12th treatments) and disappeared after the 15th treatment. Fast tremors did not usually appear until about the 3rd day of treatment. Once developed, the fast tremors, unlike slow tremors, persisted for a few days even in the absence of DFP-treatment which, however, exacerbated them. They were intermittent and were enhanced by postural changes. Pre-injection fast tremor scores were significantly greater than the control values on the 5th, 8th and 10th days. Both pre- and post-injection fast tremor scores reached peaks around the 5th day of treatment. Intense distal body tremors occurred during this period. The fast tremor scores declined over the next several days and during the third week of treatment they disappeared.

Hind-limb abduction was present only to a slight extent after the first DFP treatment, however the scores were clearly and significantly higher than pre-injection (0±0) and control values after the 3rd treatment (Fig. 4). Hind-limb abduction was not noticeable after the 5th day. Controls never displayed this behavior. Like slow tremor, hind-limb

abduction was an acute effect of DFP which disappeared a few hours after the daily treatments.

After about 5 days of treatment with DFP, the animals became hyperreactive with exaggerated startle-responses. They also exhibited ejaculating and mounting when grouped together in their original cages. Peripheral cholinergic signs disappeared during the first week of treatment.

Neurochemical Effects of DFP-Treatment

Acute treatment DA and DOPAC levels. In Experiment 1, rats were killed 0.5, 1 and 2 hr after injecting 1 mg/kg DFP. As shown in Table 1, DA levels were significantly decreased at all three time periods, the greatest change being at 1 hr (−35%) after treatment. DOPAC levels were not significantly altered at the two earlier time periods. Individual DOPAC/DA ratios (which indicate DA turn-over changes, see Discussion) were calculated and their means are represented as percentages for clarity. As can be seen, these were markedly and significantly increased.

In Experiment 2, animals were sacrificed 2 hr after giving 2 mg/kg DFP. DA was significantly decreased whereas the DOPAC level and the ratio were considerably and significantly increased.

In Experiment 3, 1 or 2 mg/kg DFP was injected 6 hr and 2 mg/kg 24 hr before sacrifice. Although there were tendencies towards decreases, neither DA nor DOPAC levels were altered significantly, (Table 1) nor were the DOPAC/DA ratios.

Chronic treatment. DA and DOPAC levels Seven or 14 days after a single dose of 2 mg/kg DFP, neither the DA nor DOPAC levels nor the ratios were found to be altered significantly (Table 2). Daily treatment with 1 mg/kg for 4 or 14 but not 28 days, produced marked and significant reductions of both DA and DOPAC levels. The DA levels were maximally decreased on the 5th day, but recovered by the 29th day. The ratios were not significantly changed.

DISCUSSION

An investigation has been made on the changes in DA metabolism in order to understand the involvement of the dopaminergic system in the behavioral tolerance to the irreversible cholinesterase inhibitor, DFP.

Behavioral Changes

Tremor [1,9], hind-limb abduction or rigidity [11] and chewing [13,39] are elicited by central muscarinic agonists given systemically or at specific brain loci. DFP also elicited these responses and during chronic treatment for 28 days, all these, except chewing, developed tolerance Even after three months of treatment chewing still occurred, becoming more intense, and both DFP- and oxotremorine-induced chewing were blocked by atropine but not methylatropine, confirming its central muscarinic origin (unpublished data). We have established for the first time that, chronic treatment with DFP causes tremors with complex characteristics. The development of tolerance for the tremors is in agreement with others [12, 23, 27]. We have, however, further shown that not all motor behaviors induced by DFP develop tolerance to it.

Striatal Dopamine and DFP Tolerance Mechanisms

The DOPAC changes, after acute treatment did not always parallel the consistently seen decreases of the DA levels, but were consistent with an increased turnover of DA [35] as the DOPAC/DA ratios were increased. Our data on the acute effects of DFP are consistent with the demonstrations that central cholinomimetics such as, oxotremorine, pilocarpine or physostigmine increased the turnover of brain DA following acute administration [6, 18, 20, 25, 38]. However, in the chronic situation we found both DA and DOPAC to be decreased with the ratios essentially unchanged. The notable decreases of DA levels in both acute and chronic situations could be partly due to a decreased synthesis of DA A report [15] suggested that chronic treatment with the organophosphates Mipafox and Leptophos also increased DA turnover However, since drugs can have complex effects on DA and DOPAC levels, the determination of the rate constant [35] would be required to establish an alteration of DA turnover by DFP treatment.

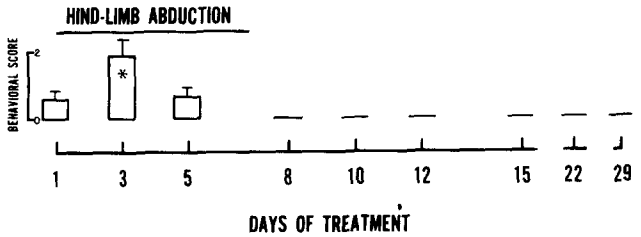

FIG. 4. Hind-limb abduction following chronic treatment with DFP *($p<0.05$). compared with pre-injection and control values using Mann-Whitney U-test

Increased cholinergic activity or diminished dopaminergic function in the basal ganglia has been implicated in the mediation of tremor [9] and rigidity [8,11] by cholinergic agonists and neuroleptics, as well as in Parkinsonism [19]. There is now much behavioral, biochemical and clinical evidence for a cholinergic-dopaminergic antagonistic interaction in the basal ganglia [3, 5, 6, 7, 8, 11, 25, 34]. A cumulative build up of ACh with repeated treatment would explain the initial phase of increases in the DFP-induced chewing, tremors and hind-limb abduction. The appreciable decrease of DA which occurred during the first week of treatment would then be expected to potentiate cholinergic behaviors such as tremors, which (fast tremors before or after injection) were actually most intense during the same period, thus supporting this hypothesis.

Reduced sensitivity to cholinergic agonists [26, 28, 29, 30] and decreased muscarinic receptor binding in DFP-tolerant rats [12, 31, 36] strongly implicate a muscarinic receptor subsensitivity in the brain. However, this mechanism alone does not explain the differential rates of development and selectivity of tolerance for behaviors induced by DFP. Recently we have [36] shown an increase of dopamine receptor numbers in the striatum, 24 hr after and 14 days after chronic treatment with DFP. The small reductions in striatal DA levels in the present experiments can only partly account for such an increase of dopamine receptors [32] A considerable lowering of brain DA leads to akinesia and rigidity [19]. In contrast, our DFP-tolerant rats were mobile and hyperreactive Hyperreactivity develops following the depletion of brain ACh [16] and is consistent with the decrease of brain ACh receptors after chronic DFP administration. Increase of DA receptors or DA turnover would partly explain the attenuation of tremors with chronic DFP treatment and is consistent with an absence of persistent motor abnormalities in the DFP-

TABLE 1

EFFECT OF ACUTE DFP-TREATMENT ON STRIATAL DA AND DOPAC LEVELS

Treatment	DA (μg/g)	DOPAC (μg/g)	$\frac{\text{DOPAC}}{\text{DA}}$ ratio (% mean)
	Experiment 1		
Control	7 31 ± 0 15	0.80 ± 0.04	100
DFP			
1 mg/kg, 0.5 hr	6.78 ± 0 11*	0.73 ± 0 03	115*
1 mg/kg, 1 hr	4.79 ± 0 36‡	0 71 ± 0 09	131†
1 mg/kg, 2 hr	5 91 ± 0.27†	0.81 ± 0.04	142‡
	Experiment 2		
Control	6 79 ± 0 27	0 78 ± 0 05	100
DFP			
2 mg/kg, 2 hr	5 29 ± 0 09†	1 20 ± 0 08‡	192‡
	Experiment 3		
Control	7.41 ± 0.88	0 70 ± 0.09	100
DFP			
1 mg/kg, 6 hr	7.27 ± 0 73	0.63 ± 0.09	108
2 mg/kg, 6 hr	7 42 ± 0 41	0.56 ± 0 08	99
2 mg/kg, 24 hr	6 06 ± 0.98	0.53 ± 0 08	109

Values are means ± S.E or their percentages on 5 animals per group.

*($p<0.05$), †($p<0$ 01) and ‡($p<0.001$) compared with the corresponding controls using Student's t-test. DA or DOPAC control values across experiments not significantly different from one another.

TABLE 2

EFFECT OF CHRONIC DFP-TREATMENT ON STRIATAL DA AND DOPAC LEVELS

Treatment	DA (μg/g)	DOPAC (μg/g)	$\frac{\text{DOPAC}}{\text{DA}}$ ratio (% mean)
Control	7 92 ± 0 39	0 69 ± 0 09	100
DFP-Single Dose			
2 mg/kg, 7 days	7 19 ± 0.87	0 53 ± 0.04	93
2 mg/kg, 14 days	6.99 ± 0 56	0 72 ± 0 07	109
DFP-Daily			
1 mg/kg, 4 days	5 41 ± 0.66*	0 46 ± 0 02*	97
1 mg/kg, 14 days	6 39 ± 0 44*	0 44 ± 0 02*	91
Control	8.46 ± 0 49	0 78 ± 0 03	100
DFP-Daily			
1 mg/kg, 28 days	8.99 ± 0.61	0 76 ± 0 04	94

Values are means ± S E. or their percentages on 4–5 animals per group

Killed 24 hr after the last dose. *($p<0.05$) compared with the control using Student's t-test Differences between DA or DOPAC control values are not significant.

tolerant rats. Therefore, these extrapyramidal dopaminergic neurochemical alterations acting in concert with the decrease of muscarinic receptors could result in the recovery of the major motor impairments such as the spontaneous tremors which can consequently disrupt feeding and shock-avoidance [30], earlier than that of the deficits involving situations with motivational (drinking) or learning (response inhibition) mechanisms [30]. The correction of the latter deficits may further involve modifications in the limbic dopaminergic or other neurotransmitter system. The apparent lack of tolerance for the non-persistent chewing-movements induced by DFP may be due to the involvement of a cholinergic-noncholinergic interaction quite different from those underlying these other behaviors and is an interesting finding which needs further investigation.

In conclusion, chronic DFP treatment leads to tolerance for some motor behaviors only and at different rates. After acute treatment the turnover of DA was increased, whereas after chronic treatment it was apparently unchanged while the levels of both DA and DOPAC were initially decreased. These DA changes, may be involved in the development of behavioral tolerance to DFP. Further biochemical and psychopharmacological studies should help to elucidate the pre- and post-synaptic transmitter mechanisms of tolerance to DFP.

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REFERENCES

1 Bebbington, A and R W Brimblecombe Muscarinic receptors in the peripheral and central nervous system *Adv Drug Res* **2:** 143–172, 1965

2 Bignami, G , N. Rosic, H. Michalek, M. Milosevic and G L Gatti. Behavioral toxicity of anticholinesterase agents In: *Behavioral Toxicology*, edited by B Weiss and V. G Laties. New York Plenum Press, 1975, pp. 155–215

3. Calne, D. B and H. L Klawans. Pathophysiology and pharmacotherapy of tremor. *Pharmacol Ther* **C2:** 113–123, 1977.

4 Carlsson, A The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacol Rev* **11:** 490–493, 1959

5 Cheney, D L., F Moroni, D Malthe-Sorenssen and E Costa. Endogenous modulators of acetylcholine turnover rate In *Cholinergic Mechanisms and Psychopharmacology*, edited by D J Jenden New York. Plenum Press, 1977, pp 551–563.

6 Corrodi, H , K Fuxe, W. Hamner, F Sjoqvist and U Ungerstedt Oxotremorine and central monoamine neurons *Life Sci* **6:** 2557–2566, 1967

7 Corrodi, H , K. Fuxe and P Lidbrink Interactions between cholinergic and catecholaminergic neurons in rat brain. *Brain Res* **43:** 397–416, 1972

8 Costall, B , R. J Naylor and J E. Olley Catalepsy and circling behavior after intracerebral injections of neuroleptic, cholinergic and anticholinergic agents into the caudate-putamen, globus pallidus and substantia nigra of rat brain *Neuropharmacology* **11:** 645–663, 1972

9 Cox, B. and D Potkonjak An investigation of the tremorigenic effects of oxotremorine and tremorine after stereotoxic injection into rat brain *Int J Neuropharmacol* **8:** 291–297, 1969

10 Davis, C S and R J. Richardson Organophosphorus compounds In *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H H Schaunberg. Baltimore Williams and Wilkins, 1980, pp 527–544

11 Dickinson, S L and P Slater Effect of lesioning dopamine noradrenaline and 5-hydroxytryptamine pathways on tremorine-induced tremor and rigidity *Neuropharmacology* **21:** 787–794, 1982

12 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

13 Ernst, A M and P G. Smelik Site of action of dopamine and apomorphine on compulsive gnawing behavior in rats. *Experientia* **22:** 837–838, 1966.

14. Fernando, J C R and G Curzon Behavioral responses to drugs releasing 5-hydroxytryptamine and catecholamines effects of treatments altering precursor concentrations in brain *Neuropharmacology* **20:** 115–122, 1981

15. Freed, V H , M A. Matin, S. C. Fang and P. P. Kar. Role of striatal dopamine in delayed neurotoxic effects of organophosphorus compounds *Eur J Pharmacol* **35:** 229–332, 1976.

16 Freeman, J J , J R. Macri, R -L Choi and D. J. Jenden Studies on the behavioral and biochemical effects of hemicholinium *in vivo* *J Pharmacol Exp Ther* **210:** 91–97, 1979

17 Fuxe, K , T. Hokfelt, L. Olson and U Ungerstedt Central monoaminergic pathways with emphasis on their relation to the so called "extrapyramidal motor system." *Pharmacol Ther 3* 169–210, 1977.

18 Haubrich, D R and W D. Reid Effects of pilocarpine or arecoline administration on acetylcholine levels and serotonin turnover in rat brain *J Pharmacol Exp Ther* **181:** 19–27, 1972.

19 Hornykiewicz, O Dopamine and brain function *Pharmacol Rev* **18:** 925–964, 1966

20 Javoy, F., Y. Agid and J. Glowinski Oxotremorine- and atropine-induced changes of dopamine metabolism in rat striatum *J Pharm Pharmacol* **27:** 677–681, 1975

21 Kozar, M D , D H Overstreet, T C Chippendale and R W Russell Changes of acetylcholinesterase activity in three major brain areas and related changes in behavior following acute treatment with diisopropylfluorophosphate *Neuropharmacology* **15:** 291–298, 1976

22. Laverty, R and K M Taylor The fluorometric assay of catecholamines and related compounds Improvements and extensions to the hydroxyindole technique *Anal Biochem* **22:** 269–279, 1968

23. Lim, D K., B. Hoskins and I K Ho. Assessment of diisopropylfluorophosphate toxicity and tolerance in rats *Res Commun Chem Pathol Pharmacol* **39:** 399–418, 1983

24 Overstreet, D H , M D Kozar and G S Lynch. Reduced hypothermic effects of cholinomimetic agents following chronic anticholinesterase treatment *Neuropharmacology* **12:** 1017–1023, 1973.

25 Perez-Cruet, J , G L Gessa, A Tagliamonte and P Tagliamonte Evidence for a balance in the basal ganglia between cholinergic and dopaminergic activity. *Fed Proc* **30:** 216, 1971

26 Russell, R W The cholinergic system search for mechanisms of action. *Annu Rev Pharmacol Toxicol* **22:** 435–463, 1982

27 Russell, R W., V G Carson, R A Booth and D J Jenden Mechanisms of tolerance to the anticholinesterase, DFP. Acetylcholine levels and dynamics in the rat brain *Neuropharmacology* **20:** 1197–1201, 1981

28 Russell, R W , D H. Overstreet, C W Cotman, V G Carson, L Churchill, F. W Dalglish and B J Vasquez. Experimental tests of hypothesis about neurochemical mechanisms underlying behavioral tolerance to the anticholinesterase diisopropylfluorophosphate. *J Pharmacol Exp Ther* **192:** 73–85, 1975

29 Russell, R. W., D H. Overstreet and R A. Netherton Sex-linked and other genetic factors in the development of tolerance to anticholinesterase DFP *Neuropharmacology* **22:** 75–81, 1983
30 Russell, R W , D. M. Warburton and D. S Segal. Behavioral tolerance during chronic changes in the cholinergic system *Commun Behav Biol* **4:** 212–228, 1969.
31. Schiller, G. D Reduced binding of ^{3}H-quinuclidinyl benzilate associated with chronically low acetylcholinesterase activity *Life Sci* **24:** 1149–1154, 1979.
32 Seeman, P Brain dopamine receptors. *Pharmacol Rev* **32:** 263–313, 1981.
33. Sethy, V H , R. H. Roth, M. J. Kuhar and M. H Van Woert. Choline and acetylcholine. regional distribution and effect of degeneration of cholinergic nerve terminals in the rat hippocampus *Neuropharmacology* **12:** 819–823, 1973
34 Sethy, V H and M H Van Woert. Regulation of striatal acetylcholine concentration by dopamine receptors. *Nature* **251:** 529–530, 1974
35. Sharman, D F. The turnover of catecholamines. In: *Central Neurotransmitter Turnover,* edited by C J. Pycock and P. V Taberner London: Croom Helm, 1981, pp. 20–58.
36. Sivam, S. P , J. C. Norris, D. K. Lim, B. Hoskins and I K Ho Influence of acute and chronic cholinesterase inhibition with diisopropylfluorophosphate on muscarinic, dopamine and GABA receptors in the rat striatum. *J Neurochem* **40:** 1414–1422, 1983
37. Weinstock, M., A. P. Zavadil and I. J. Kopin. Peripheral catecholamines mediate certain responses to central cholinergic receptor stimulation by oxotremorine In: *Monographs in Neural Sciences,* vol 7, edited by M. M Cohen Basel. Karger, 1980, pp. 138–145.
38. Westerink, B H C. and J. Korf Regional rat brain levels of 3,4-dihydroxyphenylacetic acid and homovanillic acid Concurrent fluorometric measurement and influence of drugs. *Eur J Pharmacol* **38:** 281–291, 1976
39. Wolfarth, S., E Dulska and M Lacki Comparison of the effects of the intranigral injections of cholinomimetics with systemic injections of the dopamine receptor stimulating and blocking agents in the rabbit *Neuropharmacology* **13:** 867–875, 1974.