European Journal of Pharmacology, 153 (1988) 167–173
Elsevier

EJP 50403

Chronic treatment with cholinesterase inhibitors increases α_2-adrenoceptors in rat brain

Peggie J. Hollingsworth *

Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A.

Received 4 February 1988, revised MS received 18 May 1988, accepted 31 May 1988

The specific binding of [^{3}H]clonidine to α_2-adrenoceptors on neural membranes isolated from various brain areas was determined with rats treated for 7-14 days with the cholinesterase inhibitors neostigmine, triorthocresyl phosphate (TOCP), diisopropylfluorophosphate (DFP) and paraoxon, or with vehicle. Treatment with all four inhibitors increased the number of clonidine binding sites in various brain areas. In those areas which demonstrated significant increases in [^{3}H]clonidine binding, there was also a significant inhibition of acetylcholinesterase activity. The possibility is discussed that increases in brain α_2-adrenoceptors are related to the alterations in mood seen in individuals chronically exposed to organophosphorus cholinesterase inhibitors.

α_2-Adrenoceptors; Acetylcholinesterase inhibition (chronic); [^{3}H]Clonidine binding; Brain; (Rat)

1. Introduction

More than 25 years ago Gershon and Shaw (1961) reported that farm workers in Australia who were exposed for long periods of time to organophosphorus insecticides develop a depression similar to melancholia (major depressive disorder). Subsequently, a small body of evidence has accumulated which supports the hypothesis that depression involves abnormalities of cholinergic neuronal function. The 'amine theory' of depression was generated almost simultaneously with these early observations (Bunney and Davis, 1965; Schildkraut, 1965), and subsequent biological research on depression and upon the mechanisms of action of antidepressant drugs has been dominated by those who were exploring this theory. Recently, an abnormality of prejunctional α_2-adrenoceptors has been postulated to exist in depression (Smith et al., 1981; Cohen et al., 1982). This hypothesis is based in part upon observations which suggest that antidepressant drugs and treatments cause a 'subsensitivity' of presynaptic α_2-adrenoceptors (Smith and Hollingsworth, 1983; 1984).

The development of radioligand binding techniques to identify α_2-adrenoceptors in the brain have stimulated an interest in the effects of psychotropic drugs upon the number and/or affinity of these recepors (Bylund and U'Prichard, 1983; U'Prichard, 1984). A number of studies have reported that the administration of antidepressant drugs decreases the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain (Smith et al., 1981; Vetulani, 1982; Smith and Hollingsworth, 1983). Such studies have strengthened the hypothesis that α_2-adrenoceptor function is altered in depression. Despite the postulated interaction between cholinergic and noradrenergic systems in depression (Fernando et al., 1984; Potter et al., 1985; Robinson et al., 1986), there has been no study of the possibility that the number and/or affinity of

* To whom all correspondence should be addressed: Department of Pharmacology, 0626, M6428 Medical Science I, The University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A.

α_2-adrenoceptors might be modified during long-term exposure to cholinesterase inhibitors. The purpose of the present study was to determine whether the chronic treatment of rats with various cholinesterase inhibitors might cause changes in the number and/or affinity of α_2-adrenoceptors in the rat brain. The cholinesterase inhibitors selected were TOCP, an organophosphorus compound with unusual neurotoxicity (Davis and Richardson, 1980); paraoxon, a metabolite of parathion, a commonly used insecticide, devoid of neurotoxicity (Johnson, 1975); DFP, a long-acting organophosphorus cholinesterase inhibitor; and neostigmine, a non-organophosphorus cholinesterase inhibitor. In the present study each of the inhibitors were found to increase the number of binding sites for [^{3}H]clonidine on membranes isolated from certain areas of the rat brain.

2. Materials and methods

Groups of 6 male, Sprague-Dawley rats, 170-230 g, were injected intraperitoneally (i.p.) with vehicle or with the various cholinesterase inhibitors for 7 or 14 days. The duration of treatment with any specific drug was determined by the ability of the animals to tolerate the drug. Twelve hours after the last injection, the rats were decapitated, their brains removed rapidly and placed in ice-cold Krebs physiological buffer solution and subsequently dissected according to a modification of the method described by Glowinski and Iversen (1966). The Krebs buffer consisted of (mM): NaCl 118; KCl 4.8; $CaCl_2$ 1.3; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25; disodium EDTA 0.03; glucose 10; ascorbic acid 0.06. The following regions were studied: hippocampus, amygdala, hypothalamus, parietal-occipital cortex, caudate nucleus and an area of the brainstem which contained the locus coeruleus.

2.1. Measurement of specific binding of [^{3}H]clonidine to neural membranes in rat brain homogenates

The specific binding of [^{3}H]clonidine to α_2-adrenoceptors in homogenates was measured as follows. Tissues pooled from the six rats were homogenized in 5 ml of ice-cold Tris-sucrose buffer (Tris-HCl 5 mM; sucrose 250 mM; $MgCl_2$ 1 mM; pH 7.4). The homogenates were centrifuged at $1\,100 \times g$ for 10 min, and the supernatants were saved. The supernatants were next centrifuged at $40\,000 \times g$ for 10 min, and the pellet saved. The pellet was washed twice with 2 ml of fresh incubation buffer (Tris-HCl 40 mM; $MgCl_2$ 10 mM; pH 7.5) and recentrifuged at $40\,000 \times g$ for 10 min. The final pellet was resuspended in an appropriate volume of Tris-incubation buffer. In equilibrium studies total [^{3}H]clonidine binding was measured in 1 ml aliquots of the fresh membranes which were incubated in duplicate for 30 min at 25°C with 0.5-64 nM [^{3}H]clonidine. Non-specific binding was determined by adding unlabelled clonidine, 10^{-5} M, as well as the radioligand to a second pair of incubates. Specific binding was defined as the difference between total and non-specific binding. In drug competition studies, the neural membranes were incubated with [^{3}H]clonidine at a concentration of 4 nM in the presence of concentrations of the various cholinesterase inhibitors up to and including 100 μM. Incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters and by washing with two 10 ml aliquots of Tris-incubation buffer (25°C). After termination of the incubation, the glass fiber filters were air dried and then placed in glass scintillation vials and counted for radioactivity as described by Smith et al. (1972). A computerized program (McPherson, 1983) which utilizes Scatchard (Scatchard, 1949) and Hill (Hill, 1910) transformations was used to develop preliminary estimates of the apparent dissociation constants (K_D) and the maximum number of binding sites (B_{max}) for the various radioligands. The values obtained from this program were used as preliminary estimates which are required for the non-linear regression analysis program devised by Munson and Rodbard (1980). This program, LIGAND, was used to develop the final values for K_D and B_{max}.

2.2. Measurement of acetylcholinesterase activity

Acetylcholinesterase activity was assayed by a modification of the method described by Ellman

et al. (1961). Tissues were homogenized in ice-cold, 50 mM Tris-HCl/2 mM sodium edetate buffer (pH 8.0). The volume of buffer used was sufficient to give a final concentration of 20 mg of tissue (wet weight) per ml of buffer. To assay for acetylcholinesterase activity, 0.25 ml of the homogenate together with 2.75 ml of 0.1 mM tetraisopropyl pyrophosphoramide (iso-OMPA), a specific pseudocholinesterase inhibitor, were preincubated under room air for 30 min at 37 °C. The mixture was allowed to cool to room temperature (22 °C) and placed in 3 ml quartz spectrophotometer cells. To each sample was added in sequence: 0.1 ml of color developer (10 mM 5,5′-dithio-bis(2-nitrobenzoic acid), 0.1 M Na/K phosphate, pH 7.1, and 0.44 M $NaHCO_3$) and 0.02 ml of substrate, acetylthiocholine iodide, 75 mM, and mixed. For blanks, the acetylthiocholine was replaced by 0.02 ml of distilled H_2O. The substrate and blank cells were placed in the sample and reference holders respectively of a Beckman DU-40 spectrophotometer, and the change in absorbance was recorded at 412 nm. Protein determinations were performed by the method of Lowry et al. (1951) in which bovine serum albumin was used as the protein standard.

2.3. Drugs used

The following drugs were used: acetylthiocholine iodide (Sigma), clonidine hydrochloride (Boehringer-Ingelheim), [^{3}H]clonidine hydrochloride (New England Nuclear, specific activity 41.5 mCi/mmol; radiochemical purity 98%), diisopropylfluorophosphate (Sigma); neostigmine methyl sulfate (Sigma); paraoxon (diethyl p-nitrophenyl phosphate, Aldrich); tetraisopropyl pyrophosphoramide (iso-OMPA, Sigma), triorthocresyl phosphate, (TOCP, Eastman). The paraoxon was specially purified as described by Johnson (1977). All drugs were dissolved in glass-distilled water unless otherwise specified. Triorthocresyl phosphate was diluted with corn oil (Mazola). All drug doses are expressed in terms of their salts.

3. Results

3.1. Binding of [^{3}H]clonidine to neural membranes

As reported previously (Smith et al., 1981), the regional distribution of specific binding of [^{3}H] clonidine to neural membranes was uneven (tables

TABLE 1

Effects of chronic treatment with diisopropylfluorophosphate (DFP) or triorthocresyl phosphate (TOCP) on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain. Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with diisopropylfluorophosphate (DFP), 1 mg/kg i.p., twice daily for 14 days or with thiorthocresyl phosphate (TOCP), 400 mg/kg i.p., daily for 7 days. Each point represents the mean ± S.E. of n determinations.

Brain area	Controls			DFP-treated			TOCP-treated		
	B_{max} [a]	K_D [b]	n	B_{max} [a]	K_D [b]	n	B_{max} [a]	K_D [b]	n
Amygdala	141.4 ±5.8	2.20 ±0.43	14	246.7 [e] ±16.8	1.78 ±0.05	3	187.3 [c] ±12.6	3.99 ±0.84	4
Hypothalamus	122.3 ±5.5	2.57 ±0.59	12	167.3 [c] ±24.3	3.12 ±0.65	3	121.8 ±2.9	2.35 ±0.08	4
Parietal cortex	121.8 ±5.4	2.35 ±0.27	16	179.6 [e] ±7.6	2.18 ±0.18	3	143.5 [c] ±7.0	2.86 ±0.92	4
Hippocampus	80.2 ±6.3	2.29 ±0.31	9	120.5 [d] ±8.5	2.52 ±0.21	3	118.0 [d] ±7.9	3.60 ±1.73	4
Brainstem	59.8 ±4.7	1.66 ±0.18	10	144.7 [e] ±0.5	2.38 ±0.14	3	85.2 [d] ±4.2	5.32 ±2.18	4
Caudate nucleus	52.9 ±2.7	1.94 ±0.22	13	52.7 ±9.2	1.67 ±0.22	3	77.7 [c] ±7.7	4.61 ±0.68	4

[a] fmol/mg protein, [b] nM, [c] $P < 0.05$, [d] $P < 0.005$, [e] $P < 0.00001$.

TABLE 2

Effects of chronic treatment with neostigmine or paraoxon on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain. Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with neostigmine methyl sulfate, 0.1 mg/kg i.p., twice daily for 14 days or with paraoxon, 0.1 mg/kg i.p. daily for 7 days. Each point represents the mean ± S.E. of n determinations.

Brain area	Controls			Neostigmine-treated			Paraoxon-treated		
	B_{max} [a]	K_B [b]	n	B_{max} [a]	K_D [b]	n	B_{max} [a]	K_D [b]	n
Amygdala	141.4 ±5.8	2.20 ±0.43	14	284.4 [g] ±34.7	1.84 ±0.23	4	116.6 [c] ±3.4	1.95 ±0.18	5
Hypothalamus	122.3 ±5.5	2.57 ±0.59	12	228.5 [d] ±47.1	3.96 ±0.59	4	110.0 ±6.8	2.71 ±0.35	5
Parietal cortex	121.8 ±5.4	2.35 ±0.27	16	212.2 [f] ±30.7	2.54 ±0.27	4	112.8 ±16.1	1.41 ±0.14	4
Hippocampus	80.2 ±6.3	2.29 ±0.31	9	123.9 ±22.3	3.43 ±0.52	4	81.7 ±4.8	2.52 ±0.26	5
Brainstem	59.8 ±4.7	1.66 ±0.18	10	128.7 [f] ±15.1	2.41 ±0.19	4	92.0 [g] ±4.8	2.38 ±0.38	5
Caudate nucleus	52.9 ±2.7	1.94 ±0.22	13	114.5 [f] ±18.4	2.78 ±0.59	4	52.2 ±0.9	2.27 ±0.26	5

[a] fmol/mg protein, [b] nM, [c] $P < 0.05$, [d] $P < 0.01$, [e] $P < 0.005$, [f] $P < 0.0005$, [g] $P < 0.0001$.

1 and 2). There were no differences between rats treated with corn oil and those treated with saline. Therefore, the results from both groups were pooled. For rats treated with vehicle alone, the highest degree of binding occurred with membranes from the amygdala, hypothalamus and parietal cortex. An intermediate degree of binding occurred with membranes from the hippocampus, and the lowest binding was found with membranes from the brainstem and caudate nucleus. Non-specific binding ranged from 5% in the amygdala and parietal cortex to 14% for the caudate nucleus. None of the drug treatments altered the degree of non-specific binding to membranes from any brain area. When added in vitro in concentrations up to 100 μM none of the cholinesterase inhibitors used in this study modified the specific binding of [^{3}H]clonidine to neural membranes.

Treatment of rats with DFP, 1 mg/kg i.p., twice daily for 15 days significantly increased the specific binding of [^{3}H]clonidine to membranes from the amygdala (table 1, 74.5%, $P < 0.00001$), hypothalamus (36.8%, $P < 0.05$), parietal cortex (47.5%, $P < 0.00001$), hippocampus (50.2%, $P < 0.005$) and brainstem (142%, $P < 0.00001$).

Chronic TOCP treatment also increased significantly the binding of [^{3}H]clonidine to neural membranes isolated from all areas of the rat brain except the hypothalamus (table 1). The largest increases occurred with membranes isolated from the hippocampus (47.1%, $P < 0.005$), caudate nucleus (46.9%, $P < 0.05$) and brainstem (42.5%, $P < 0.005$). The smallest increase occurred with membranes from the parietal cortex (17.8%, $P < 0.05$).

Of the various cholinesterase inhibitors, neostigmine, the only non-organophosphorus inhibitor studied, caused the largest increases in [^{3}H]clonidine binding, and statistically significant increases occurred with membranes from all areas except the hippocampus (table 2). The largest increases occurred with membranes isolated from the caudate nucleus (116.5%, $P < 0.0005$), brainstem (115.2%, $P < 0.0005$) and amygdala (101.1%, $P < 0.0001$). Increases in binding of intermediate magnitude occurred with membranes from the hypothalamus (86.8%, $P < 0.01$) and parietal cortex (74.2%, $P < 0.0005$). Although binding to membranes from the hippocampus increased by 54.5%, this increase did not quite meet statistical significance.

In contrast to treatment with the other three cholinesterase inhibitors, chronic administration of paraoxon increased binding of [^{3}H]clonidine only to membranes isolated from the brainstem

(53.9%, $P < 0.0001$). No significant changes were observed with membranes from the other brain areas with the exception of the amygdala where a small, but significant decrease in binding occurred (17.5%, $P < 0.05$).

None of the treatments produced a significant change in the K_D for the binding of [^{3}H]clonidine to membranes from any brain area.

3.2. Inhibition of acetylcholinesterase activity

The distribution of acetylcholinesterase activity in the various regions of the rat brain was very similar to that reported by Bennett et al. (1966) who measured hydrolysis of acetylcholine and by Wade and Timiras (1980) who measured the hydrolysis of acetylthiocholine in homogenates. Both DFP and TOCP had similar effects upon acetylcholinesterase activity in the rat brain (table 3), although the degree of inhibition varied with the brain region. Long-term administration of DFP or TOCP markedly inhibited acetylcholinesterase activity in homogenates from the caudate (78.2%, $P < 0.0001$, and 84.8%, $P < 0.0005$, respectively), amygdala (77.5%, $P < 0.01$, and 68.7%, $P < 0.0005$, respectively), hippocampus (77.7%, $P < 0.0001$, and 72.1%, $P < 0.0005$) and parietal cortex (80.3%, $P < 0.0001$, and 69.5%, $P < 0.005$, respectively). A lesser degree of inhibition was produced by DFP and TOCP in homogenates from the brainstem (52.8%, $P < 0.001$, and 50.7%, $P < 0.0005$, respectively) and the hypothalamus (56.8%, $P < 0.01$, and 30.3%, $P < 0.025$, respectively). Chronic treatment with neostigmine also produced a statistically significant inhibition of acetylcholinesterase activity in homogenates from all brain areas with the exception of the hippocampus (table 3). In contrast, long-term treatment with paraoxon significantly decreased acetylcholinesterase activity only in homogenates from the brainstem (26.3%, $P < 0.05$) and the caudate nucleus (41.7%, $P < 0.005$). An attempt was made to treat rats with a higher dose of paraoxon, namely 0.3 mg/kg i.p. daily, but the rats convulsed and died with this dose within 15 min of either the third or fourth injection.

4. Discussion

Gershon and Shaw (1961) provided the first suggestion that cholinergic neuronal dysfunction might be involved in the pathogenesis of depressive disorders. Subsequently, a small body of evidence accumulated which supported the hypothesis that depression involves abnormalities of cholinergic function. The acute administration of the centrally acting cholinesterase inhibitor, physostigmine, will precipitate a depressive episode when administered to individuals with affective disorders (Janowsky et al., 1972; 1974; 1981), to subjects acutely intoxicated with marijuana (El-Yousef et al., 1973), as well as to certain 'normal' volunteers (Bowers et al., 1964). In an early study in which a variety of central and

TABLE 3

Acetylcholinesterase activity [a] in various areas of the rat brain before and after long-term treatment with cholinesterase inhibitors.

Brain area	Control [b]	DFP [c]	Neostigmine [d]	TOCP [e]	Paraoxon [f]
Caudate nucleus	149.8 ± 8.3	32.7 ± 5.1 [m]	109.3 ± 9.3 [h]	22.8 ± 3.7 [l]	87.4 ± 12.5 [j]
Amygdala	76.0 ± 7.4	17.1 ± 2.9 [i]	49.3 ± 4.2 [g]	23.8 ± 2.9 [l]	62.6 ± 5.0
Brainstem	50.6 ± 2.5	23.9 ± 1.7 [k]	37.1 ± 4.8 [h]	24.9 ± 3.7 [l]	37.3 ± 7.2 [g]
Hypothalamus	31.7 ± 2.2	13.7 ± 1.9 [i]	24.1 ± 2.0 [g]	22.1 ± 2.4 [h]	24.3 ± 6.7
Hippocampus	30.5 ± 1.9	6.8 ± 1.0 [m]	28.0 ± 3.2	8.5 ± 0.7 [l]	25.5 ± 3.7
Parietal	25.9 ± 1.7	5.1 ± 0.4 [m]	17.7 ± 1.3 [h]	7.9 ± 0.6 [j]	21.8 ± 4.9

[a] nmol acetylthiocholine hydrolyzed/min per mg protein. [b] Eleven control rats were treated with vehicle alone for 7 to 14 days. [c] Three rats were treated with diisopropylfluorophosphate, 1 mg/kg i.p. twice daily for 14 days. [d] Four rats were treated with neostigmine methyl sulfate, 0.1 mg/kg i.p. twice daily for 14 days. [e] Three rats were treated with triorthocresyl phosphate, 400 mg/kg i.p., daily for 7 days. [f] Three rats were treated with paraoxon, 0.1 mg/kg i.p. daily for 7 days. Each point represents the mean of n determinations ± S.E. [g] $P < 0.05$, [h] $P < 0.02$, [i] $P < 0.01$, [j] $P < 0.005$, [k] $P < 0.001$, [l] $P < 0.0005$, [m] $P < 0.0001$.

peripheral effects of DFP were compared in schizophrenic and manic-depressive subjects, the patients with affective disorders were found to be more sensitive to both the muscarinic and nicotinic effects of this organophosphorus cholinesterase inhibitor than were the schizophrenic patients (Rowntree et al., 1950). Finally, it has been noted that the many tricyclic antidepressant drugs which are efficacious in the treatment of depressive disorders have prominent anticholinergic activity (Sulser et al., 1964).

Numerous studies have shown that the tricyclic antidepressants as well as other treatments for depression alter α_2-adrenoceptors. Various investigators have reported that the administration of tricyclic antidepressants increases (Garcia-Sevilla et al., 1980; Johnson et al., 1980; Reisne et al., 1982), decreases (Smith et al., 1981; 1983; Vetulani, 1982) or fails to change (Sugrue, 1982; Sethy et al., 1983) the specific binding of [^{3}H]clonidine to neural membranes isolated from the rat brain. The differences in the changes in clonidine binding which were reported can be attributed to differences in drug doses, duration of drug administration and the brain areas which were studied. The relationship of these variables to changes in α_2-adrenoceptor density have been reviewed (Smith and Hollingsworth, 1984). There has been no previous attempt, however, to evaluate the effects of long-term exposure to cholinesterase inhibitors to changes in the number and/or affinity of α_2-adrenoceptors in the brain.

The present study demonstrates that long-term administration of cholinesterase inhibitors results in an increase in the number of α_2-adrenoceptors found in specific areas of the rat brain. This increase in receptor density is qualitatively, if not quantitatively, associated with inhibition of acetylcholinesterase. TOCP increased the specific binding of [^{3}H]clonidine to membranes from all brain areas except the hypothalamus. Although it inhibited acetylcholinesterase in all brain areas, the effect was least in the hypothalamus. DFP inhibited acetylcholinesterase activity in all areas which were studied and increased clonidine binding in all areas with the exception of the caudate nucleus. Neostigmine inhibited acetylcholinesterase activity in all areas except the hippocampus and significantly increased [^{3}H]clonidine binding to membranes from all areas except the hippocampus. Finally, paraoxon under the present experimental conditions only inhibited acetylcholinesterase activity in the brainstem and caudate nucleus and significantly increased binding of clonidine to membranes isolated from the brainstem. Thus, there appears to be a fairly good qualitative correlation between inhibition of acetylcholinesterase activity and increases in the density of α_2-adrenoceptors in various areas of the brains of rats exposed chronically to cholinesterase inhibitors. The present study suggests that drugs and procedures which produce depression might also produce changes in the number of α_2-adrenoceptors in the central nervous system. The functional significance of the receptor changes observed in the present study remain to be elucidated.

Acknowledgements

Supported by U.S. Public Health Service Grants ES-03490 and MH-36226.

References

Bennett, E.L., M.C. Diamond, H. Morimoto and M. Herbert, 1966, Acetylcholinesterase activity and weight measures in fifteen brain areas from six lines of rats, J. Neurochem. 13, 563.

Bowers, M.B., E. Goodman and V.M. Sim, 1964, Some behavioral changes in man following anticholinesterase administration, J. Nerv. Ment. Dis. 138, 383.

Bunney, W.E. and J.M. Davis, 1965, Norepinephrine in depressive reactions, Arch. Gen. Psychiat. 13, 483.

Bylund, D.B. and D.C. U'Prichard, 1983, Characterization of alpha$_1$- and alpha$_2$-adrenergic receptors, Int. Rev. Neurobiol. 24, 343.

Cohen, R.M., I.C. Campbell, M. Dauphin, J.F. Tallman and D.L. Murphy, 1982, Changes in α- and β-receptor densities in rat brain as a result of treatment with monoamine oxidase inhibiting antidepressants, Neuropharmacology 21, 293.

Davis, C.S. and R.J. Richardson, 1980, Organophosphorus compounds, in: Clinical and Experimental Neurotoxicology, Chapter 36, eds. P.S. Spencer and H.H. Schaumberg (Williams and Wilkins, Baltimore) p. 527.

Ellman, G.L., K.D. Courtney, V. Andres, Jr. and R.M. Featherstone, 1961, A new and rapid colorimetric de-

termination of acetylcholinesterase activity, Biochem. Pharmacol. 7, 88.

El-Yousef, M.K., D.S. Janowsky, J.M. Davis and J.E. Rosenblatt, 1973, Induction of severe depression by physostigmine in marijuana intoxicated individuals, Br. J. Addict. 68, 321.

Fernando, J.C.R., B. Hoskins and I.K. Ho, 1984, Effect on striatal dopamine metabolism and differential motor behavioral tolerance following chronic cholinesterase inhibition with diisopropylfluorophosphate, Pharmacol. Biochem. Behav. 20, 951.

Garcia-Sevilla, J.A., C.B. Smith, L. Chapelsky and P.J. Hollingsworth, 1980, Long-term desipramine treatment increases ^{3}H-clonidine binding in various rat brain regions, Fed. Proc. 39, 1097.

Gershon, S. and F.H. Shaw, 1961, Psychiatric sequelae of chronic exposure to organophosphorus insecticides, Lancet 1, 1371.

Glowinski, J. and L.L. Iversen, 1966, Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-dopa in various regions of the brain, J. Neurochem. 13, 655.

Hill, A.V., 1910, The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves, J. Physiol. 40, 4.

Janowsky, D.S., M.K. El-Yousef and J.M. Davis, 1974, Acetylcholine and depression, Psychosomat. Med. 36, 248.

Janowsky, D.S., M.K. El-Yousef, J.M. Davis and J.J. Sekerke, 1972, A cholinergic-adrenergic hypothesis of mania and depression, Lancet 1, 632.

Janowsky, D.S., S.C. Risch, L.L. Judd, L.Y. Huey and D.C.P. Parker, 1981, Cholinergic supersensitivity in affect disorder patients: behavioral and neuroendocrine observations, Psychopharmacol. Bull. 17, 129.

Johnson, M.K., 1975, Organophosphorus esters causing delayed neurotoxic effects. Mechanism of action and structure/activity studies, Arch. Toxicol. 34, 259.

Johnson, R.W., T. Reisne, S. Spotnitz, N. Wiech, R. Ursillo and H.I. Yamamura, 1980, Effects of desipramine and yohimbine on α_2- and β-adrenoreceptor sensitivity, European J. Pharmacol. 67, 123.

Lowry, O.H., N.F. Rosebrough, A.G. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.

McPherson, G.S., 1983, A practical computer-based approach to the analysis of radioligand binding experiments, Comput. Prog. Biomed. 17, 107.

Munson, P.J. and D. Rodbard, 1980, LIGAND: a versatile computerized approach for characterization of ligand-binding systems, Anal. Biochem. 107, 220.

Potter, P.E., M. Hadjiconstantinou, J.S. Rubenstein and N.H. Neff, 1985, Chronic treatment with diisopropylfluorophosphate increases dopamine turnover in the striatum of the rat, European J. Pharmacol. 106, 607.

Reisne, T., R. Johnson, N. Wiech, R. Ursillo and H.I. Yamamura, 1982, Rapid desensitization of central beta-receptors and up-regulation of alpha$_2$-receptors following antidepressant treatment, in: Typical and Atypical Antidepressants: Molecular Mechanisms, eds. E. Costa and G. Racagni (Raven Press, New York) p. 63.

Robinson, S.E., M.A. Rice and K.L. Hambrecht, 1986, Effect of intrastriatal injection of diisopropylfluorophosphate on acetylcholine, dopamine and serotonin metabolism, J. Neurochem. 46, 1632.

Rowntree, D.W., S. Nevin and A. Wilson, 1950, The effects of diisopropylfluorophosphonate in schizophrenia and manic depressive psychosis, J. Neurol. Neurosurg. Psychiat. 13, 47.

Scatchard, G., 1949, The attractions of proteins for small molecules and ions, Ann. N.Y. Acad. Sci. 51, 660.

Schildkraut, J.J., 1965, The catecholamine hypothesis of affective disorders: a review of supporting evidence, Am. J. Psychiat. 122, 509.

Sethy, V.H., R.W. Carlsson and D.W. Harris, 1983, Effect of chronic antidepressant treatment on a$_2$-adrenoceptors, Drug Dev. Res. 3, 287.

Smith, C.B., J.A. Garcia-Sevilla and P.J. Hollingsworth, 1981, Alpha$_2$-adrenoceptors in rat brain are decreased after long-term tricyclic antidepressant drug treatment, Brain Res. 210, 413.

Smith, C.B. and P.J. Hollingsworth, 1983, Alpha$_2$-adrenoreceptors and the mechanism of action of tricyclic antidepressant drugs, in: Neuronal and Extraneuronal Events in Autonomic Pharmacology, ed. W.W. Fleming, S.Z. Langer, K.-H. Graefe and N. Weiner (Raven Press, New York) p. 181.

Smith, C.B. and P.J. Hollingsworth, 1984, Alpha$_2$-adrenoreceptors and antidepressant treatments, in: Proceedings, IUPHAR 9th International Congress of Pharmacology, London, Vol. 3, eds. W. Paton, J. Mitchell and P. Turner (The MacMillan Press, Ltd., London) p. 131.

Smith, C.B., M.J. Sheldon, J.H. Bednarczyk and J.E. Villarreal, 1972, Morphine-induced increases in the incorporation of ^{14}C-tyrosine to ^{14}C-dopamine and ^{14}C-norepinephrine in the mouse brain, J. Pharmacol. Exp. Ther. 180, 547.

Sugrue, M.F., 1982, Effect of chronic antidepressant treatment on rat brain α_2-adrenoceptor sensitivity, in: Typical and Atypical Antidepressants. Molecular Mechanisms, eds. E. Costa and G. Racagni (Raven Press, New York) p. 55.

Sulser, F., M.H. Bickel and B.B. Brodie, 1964, The action of desmethylimipramine in counteracting sedation and cholinergic effects of reserpine-like drugs, J. Pharmacol. Exp. Ther. 144, 321.

U'Prichard, D.C., 1984, Biochemical characteristics and regulation of brain alpha$_2$-adrenoceptors, Ann. N.Y. Acad. Sci. 430, 55.

Vetulani, J., 1982, Adaptive changes as the mode of action of antidepressant treatments, in: Typical and Atypical Antidepressants: Molecular Mechanisms, eds. E. Costa and G. Racagni (Raven Press, New York) p. 27.

Wade, P.D. and P.S. Timiras, 1980, A regional study of the molecular forms of acetylcholinesterase in the brain of developing and adult rats, Dev. Neurosci. 3, 101.