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Effects of Psychotomimetic Compounds on Human Pseudocholinesterase

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ABSTRACT

FRIED, GEORGE H. AND WILLIAM ANTOPOL. *Effects of psychotomimetic compounds on human pseudocholinesterase.* J. Appl. Physiol. 11(1): 25 28. 1957. The effects of serotonin, lysergic acid diethylamide (LSD), chlorpromazine, and reserpine on serum cholinesterase were determined manometrically. Inhibitions of 50% were obtained with 2×10^{-3} M serotonin, 10^{-6} M LSD, and 5×10^{-5} M chlorpromazine. Reserpine had no measurable effect. Marked potentiation of enzyme activity was achieved with extremely low concentrations of the three drugs (30% potentiation with 5×10^{-6} M serotonin, 50% potentiation with 6×10^{-9} M LSD, and 25% potentiation with 5×10^{-7} M chlorpromazine). This potentiation above control values may be of significance in explaining some central effects of these psychotomimetic agents. It is also felt that both potentiation and inhibition may be related to tertiary amine groupings on the inhibitor molecule.

ATTEMPTS to establish a biochemical basis for psychic disturbances have been stimulated by recent discoveries of the effects of hallucinogenic and tranquilizing indoles on isolated tissue responses (1) and on affective behavior (2). Interest initially centered on the role of serotonin (5-hydroxytryptamine), a natural constituent of nerve tissue, in mediating effects of hallucinogens and tranquilizers since these latter psychotomimetic substances could alter tissue levels of serotonin in the organism or antagonize its activity (3). However, more recent investigations indicate that psychotomimetic compounds do not exert their effects through altered serotonin levels but probably act in a more complex manner (4).

The present investigations deal with the influence of psychotomimetic compounds on pseudocholinesterase. Pseudocholinesterase (ChE) differs from 'true' acetylcholinesterase (AChE) in that it is less specific for the substrate acetylcholine (ACh), i.e. it shows greater activity with butyrylcholine than with ACh, demonstrates no inhibition of enzyme activity at higher substrate concentrations and attacks benzoylcholine while demonstrating no activity with acetyl-beta-methylcholine. The presence of ChE has been demonstrated in the central nervous system, particularly in glial cells and sensory ganglia (5), and there is some indication in the literature of the sensitivity of this enzyme to several psychotomimetic compounds (6, 7).

Human serum was used as the enzyme source since it is thought to contain a single ChE (8), identical with or closely similar to the corresponding enzyme found in the nervous system (9). Compounds tested for anticholinesterase activity were serotonin (serotonin creatinine sulfate, Mann), chlorpromazine hydrochloride (Thorazine:HCl, S.K.F.), reserpine (reserpine phosphate, Ciba), and lysergic acid diethylamide (LSD_{25}, Sandoz).

METHODS

ChE activity was determined manometrically in a Warburg constant volume respirometer by a modification of the method of Ammon (10), which involves estimation of the volume of carbon dioxide evolved from a bicarbonate containing system by the acetic acid formed in the splitting of ACh, with the system buffered against carbon dioxide. Acetylcholine chloride (Matheson) was employed as substrate. Serum samples obtained from individuals free of diseases known to

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TABLE 1. EFFECTS OF PSYCHOTOMIMETIC COMPOUNDS ON PSEUDOCHOLINESTERASE

Average Conc. *M*	No. of Determ.	Average Activity *Cont.*	*Expt.*	Inhibition %
		Serotonin		
3.0×10^{-7}	1	37	37	
5.0×10^{-6}	5	33	44	−31 ± 5.2
3.0×10^{-5}	4	33	41	−25 ± 12.3
5.0×10^{-5}	2	34	36	
4.0×10^{-4}	4	31	29	
1.0×10^{-3}	2	51	28	46 ± 1.4
4.0×10^{-3}	4	33	14	59 ± 4.4
		LSD		
4.7×10^{-9}	4	23	34	−45 ± 10.4
4.0×10^{-8}	3	41	40	
6.0×10^{-7}	3	44	30	32 ± 1.3
1.3×10^{-6}	3	41	12	70 ± 4.7
6.0×10^{-6}	2	51	10	82 ± 1.0
		Chlorpromazine		
3.5×10^{-7}	3	36	45	−24 ± 1.7
1.0×10^{-6}	1	35	39	−12
7.5×10^{-6}	2	37	27	26 ± 15.5
5.0×10^{-5}	3	37	17	54 ± 5.8

alter serum ChE levels were diluted 1:50 with bicarbonate buffer. At these dilute enzyme levels inhibition becomes a function of the specific concentration of the inhibitor alone (11), so that slight variations in enzymatic activity of different serum samples should not change the degree of inhibition encountered. Reagents and drugs were also made up in bicarbonate buffer. Since the drugs used were not esters they did not undergo hydrolysis.

Drugs (0.5 ml) and diluted serum (0.5 ml) were pipetted directly into the main compartment of the flasks, while ACh (1.0 ml) was added to the sidearm to give a final substrate concentration of 0.02 M. All flasks were adjusted to a final fluid volume of 2.0 ml with buffer and then flushed with 95% N_2–5% CO_2 for 10 minutes. The initial pH was about 7.6, and this value did not appear to change by more than several tenths in either direction during the course of the reaction. Substrate was tipped into the main compartment and the flasks equilibrated for 10 minutes at a bath temperature of 30°C. Readings were taken every 10 minutes. Calculations of the velocity constants of cholinesterase activities were based on microliters of CO_2 liberated during the 1st hour, with cumulative values for each 10-minute period also plotted against time. There was no appreciable diminution in the rate of hydrolysis in control flasks containing substrate and enzyme alone during the experimental period. Spontaneous hydrolysis of ACh, both with and without the drug being tested, was determined for each experiment by substituting buffer for the diluted serum. These values were subtracted from corresponding control and experimental readings so that the cited results represent enzymatic activity alone.

Reserpine was insoluble at concentrations greater than 2×10^{-5}M, while chlorpromazine was apparently insoluble at concentrations higher than 10^{-4}M. Serotonin and LSD were quite soluble.

RESULTS

The I_{50} (concentration of inhibitor causing 50% inhibition) for serotonin was approximately 2×10^{-3} M, while levels of 5×10^{-5} M were without effect upon esterase activity. For LSD, the I_{50} was of the order of 10^{-6} M, with no effects evident at 10^{-8} M. With chlorpromazine, the I_{50} was approximately 5×10^{-5} M, while concentrations ranging from 1×10^{-6} M to 5×10^{-6} M had only negligible effects (table 1). Reserpine did not inhibit at concentrations up to its maximum solubility.

Marked potentiation of enzyme activity was observed with very low concentrations of the inhibiting compounds (negative values in table 1). For serotonin, concentrations of 5×10^{-6} M elicited an average augmentation of 30%. A 50% augmentation was produced by 6×10^{-9} M LSD, while potentiations of about 25% occurred with chlorpromazine concentrations approximating 10^{-7} M.

The inhibition of ChE by all three drugs was easily reversed with excess substrate, in agreement with previous findings, indicating the competitive nature of this inhibition (7, 12).

While straight line relationships were generally obtained for enzymatic activities with time in the case of controls and with levels of drugs causing potentiation, the curves in cases of inhibition were often erratic. This is illustrated in figure 1.

There was some variation in drug effects with different serum samples, particularly in cases of potentiation. Data on potentiation afforded by levels of LSD of 6×10^{-9} M were obtained from serum samples with esterase activity constants of 18, 20, 24 and 29. Corresponding potentiation percentages were 72, 60, 38 and 38, which suggests enhanced potentiation with serum of depressed esterase activity. ChE levels in the serum samples utilized generally varied between 30 and 40 μl CO_2 liberated per hour, well within the range encountered in healthy normal subjects.

In several experiments tryptamine appeared to exert inhibitory effects of the same order as serotonin, while tyramine and several tryptophane derivatives demonstrated little or no

activity. Hydroxyindoleacetic acid did not inhibit at concentrations as high as 10^{-3} M, but exhibited a variable potentiation at 6×10^{-4} M.

DISCUSSION

Our findings in the cases of inhibition are in substantial agreement both with those of Thompson *et al.* (7) on the inhibition of ChE by LSD and with the work of Erspamer (12) on the inhibitory action of serotonin. A similarity in the inhibitory effects of tryptamine and serotonin was also noted by this latter investigator. With chlorpromazine, Erdös *et al.* (13) obtained a ChE I_{50} value of 3×10^{-5} M, which approximates the present finding. However, since the free base of chlorpromazine is virtually insoluble at pH values above 6 (personal communication, Smith, Kline and French Laboratories), these I_{50} values may be too high. None of these investigators reported potentiation of enzyme activity at lower drug concentrations. Since these lower concentrations may be closer to the physiological levels of such naturally occurring indoles as serotonin, the potentiation observed here may be of greater significance than the inhibition. The transient increases in cholinesterase activity with both serotonin and LSD noted by Tonini (14, 15) are not comparable with the present findings because those results were obtained from whole brain homogenates containing a mixture of esterases.

Although both ChE and AChE are now believed to possess an anionic as well as an esteratic site (16), the tertiary amines apparently act as powerful inhibitors of ChE while quarternary ammonium bases are more specific inhibitors of AChE (17). Since LSD possesses three tertiary amine groups and was also most potent in its inhibiting and potentiating effects, it is reasonable to suppose that these groupings may be involved in the two actions. Both effects may even be produced in a similar manner, possibly through some binding at the anionic site. Physostigmine, another powerful inhibitor of ChE, also contains three tertiary amine groups. Bufotenine, a hallucinogen shown to inhibit ChE (6), contains two tertiary amine groups, as does serotonin, chlorpromazine and tryptamine. Tyramine, with only one such grouping, is without effect. However, the

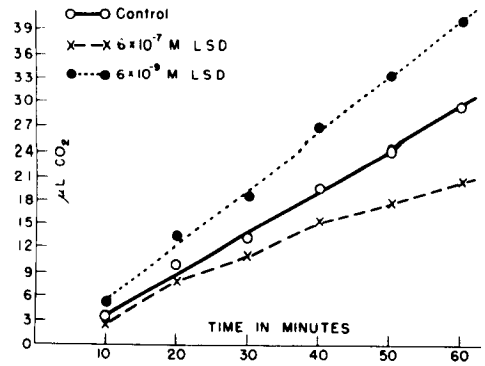

FIG. 1. Effect of varying concentrations of lysergic acid diethylamide (LSD) on cholinesterase activity.

absence of any noticeable effect with several tryptophane derivatives, which also possess two of these amine groupings, points to the difficulty of characterizing the exact mechanism of drug-enzyme interaction at present.

The definitive role of ChE is unknown. However, the reported alteration in levels of this enzyme in intestine and nerve during functional disorders of these structures (18, 19) and the alterations in serum ChE encountered in hepatic diseases and hyperthyroidism (20) suggest some key function similar to that of the true AChE. Thus it has been suggested (21) than cholinesterase serves as a defense mechanism by destroying excess ACh. It has also been shown that in thiamin-deficient pigeons the onset of polyneuritic symptoms is associated with a rise in cholinesterase (22).

The present observations that both serotonin and LSD may inhibit or potentiate ChE, depending on concentration, may partially explain the similarity in action of these 2 compounds in some cases and the antagonisms evidenced in others (23). There is, then, an indication that the influence of psychotomimetic compounds on ChE activity is not fortuitous but may explain certain effects of these drugs. This is particularly applicable in the case of the oligodendroglia, structures rich in ChE, which have been shown to undergo prolonged contraction under the influence of serotonin (24). Although reserpine failed to influence ChE directly it is believed to exert its effects through the release of serotonin (25), so that this tranquilizing agent also may act, albeit indirectly, through an alteration of ChE levels.

Whether naturally occurring psychic defects may be mediated in part through alteration of ChE activity is conjectural at this point, but the demonstrated presence of the enzyme in the brain (3) and its sensitivity to psychotomimetic substances suggest such a possibility.

REFERENCES

1. SHAW, E. AND D. W. WOOLLEY. *J. Pharmacol. & Exper. Therap.* 111: 43, 1954.
2. SLOANE, B. AND J. W. L. DOUST. *J. Ment. Sc.* 100: 129, 1954.
3. AMIN, A. H., T. B. B. CRAWFORD AND J. H. GADDUM. *J. Physiol.* 126: 596, 1954.
4. CERLETTI, A. AND E. ROTHLIN. *Nature* 176: 785, 1955.
5. HEBB, C. O. *Pharmacol. Rev.* 6: 30, 1954.
6. SOBOTKA, H. AND W. ANTOPOL. *Enzymologia* 4: 180, 1937.
7. THOMPSON, R. H. S., A. TICKNER AND G. R. WEBSTER. *Brit. J. Pharmacol.* 10: 61, 1955.
8. LEVINE, M. G. AND A. A. SURAN. *Enzymologia* 15: 17, 1952.
9. MYERS, D. K. *Biochem. J.* 62: 556, 1956.
10. AMMON, R. *Arch. ges. Physiol.* 233: 486, 1933.
11. KOELLE, G. B. AND A. GILMAN. *J. Pharmacol. & Exper. Therap.* 95: 166, 1949.
12. ERSPAMER, V. *Pharmacol. Rev.* 6: 425, 1954.
13. ERDÖS, E. G., F. F. FOLDES, N. BAART AND S. P. SHANOR. *Fed. Proc.* 15: 420, 1956.
14. TONINI, G. *Boll. Soc. ital. biol. sper.* 31: 700, 1955.
15. TONINI, G. *Boll. Soc. ital. biol. sper.* 31: 708, 1955.
16. SHUKAVA, R. AND M. SHINODA. *J. Biochem.* 43: 315, 1956.
17. AUGUSTINSSON, K. B. *Acta physiol. scandinav.* 15: Suppl. 15, 1948.
18. KOELLE, G. B., E. J. KOELLE AND J. S. FRIEDENWALD. *J. Pharmacol. & Exper. Therap.* 100: 180, 1950.
19. EARL, C. J. AND R. H. S. THOMPSON. *Brit. J. Pharmacol.* 7: 685, 1952.
20. ANTOPOL, W., L. TUCHMAN AND A. SCHIFRIN. *Proc. Soc. Exper. Biol. & Med.* 36: 46, 1937.
21. ANTOPOL, W., S. GLAUBACH AND D. GLICK. *Proc. Soc. Exper. Biol. & Med.* 42: 280, 1930.
22. ANTOPOL, W., S. GLAUBACH AND D. GLICK. *Proc. Soc. Exper. Biol. & Med.* 42: 670, 1930.
23. SHAW, E. AND D. W. WOOLLEY. *Science* 124: 121, 1956.
24. BENITEZ, H. H., M. R. MURRAY AND D. W. WOOLLEY. *Anat. Rec.* 121: 466, 1955.
25. SHORE, P. A., S. L. SILVER AND B. B. BRODIE. *Science* 122: 284, 1955.