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The Effect of Several Psychotomimetic Drugs on Human Serum Cholinesterase

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With 4 Figures in the Text

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Several drugs reported to cause psychotomimetic symptoms in man inhibit human serum cholinesterase. The most potent of the psychotomimetic drugs, lysergic acid diethylamide (LSD), at 10^{-6} M produces 50 per cent inhibition of hydrolysis of several aliphatic substrates (THOMPSON et al. 1955). Bromlysergic acid (BOL), ineffective as a psychotomimetic agent (CERLETTI and ROTHLIN 1955) inhibits serum cholinesterase almost as effectively as does LSD with benzoylcholine (BzCh) and acetylcholine (ACh) as substrates, measured manometrically (ZEHNDER and CERLETTI 1956). FRIED and ANTAPOL (1957) confirmed this inhibitory effect of LSD but reported that lower concentrations (10^{-9} M) activated human serum cholinesterase, as measured manometrically with ACh as substrate.

Among the drugs considered by EVARTS (1957) to meet his criteria as psychotomimetic agents: diisopropylfluorophosphonate (DFP) (MASUR and BODANSKY 1946) and tetraethylpyrophosphate (TEPP) (MANGUN and DUBOIS 1947) are among the most potent serum cholinesterase inhibitors known; nalorphine inhibits horse (BLOHM and WILLMORE 1951) but not dog (YOUNG et al. 1955) serum cholinesterase, while the only report of its effect on human serum cholinesterase is of activation (FOLDES et al. 1957 a). Mescaline and adrenochrome are weak inhibitors of human serum cholinesterase (GOLDENBERG and GOLDENBERG 1956) when ACh is used as substrate. No reports of the effects of harmine and of tetrahydrocannabinol on serum cholinesterase have been noted.

In most of these studies ACh was used as substrate. BzCh hydrolysis by serum cholinesterase differs from ACh hydrolysis both kinetically (AUGUSTINSSON 1950; KALOW et al. 1956) and in sensitivity to various inhibitors (FOLDES et al. 1957 b). The theoretical advantages of testing the effect of inhibitors on dilute (5×10^{-5} M) solution of BzCh have been discussed by KALOW et al. (1956). Also, the occurrence of a genetically determined atypical form of human serum cholinesterase which differs

from normal in its affinity for certain inhibitors and substrates has been demonstrated (KALOW and GENEST 1957; KALOW and DAVIES 1959).

This study was designed primarily to provide directly comparable data on the cholinesterase inhibiting properties of several psychotomimetic drugs obtained under similar standardized conditions. Both typical and atypical enzymes have been tested in so far as the available supply of the latter permitted[1].

From the psychotomimetic agents listed by EVARTS (1957), tetraethylpyrophosphate was not available for study, adrenochrome was excluded because its extreme instability makes it unsatisfactory for inclusion (GREEN and RICHTER 1937), and tetrahydrocannabinol was excluded because of its limited solubility. Three recently introduced psychotomimetics have been included: 2-diethylaminoethyl cyclopentyl (2 thienyl) glycolate hydrochloride, designated WIN 2299 (PENNES and HOCH 1957), N,N-dimethyltryptamine (ST. SZARA 1956; SAI-HALÁSZ et al. 1958), and Psilocybin (HOFMANN et al. 1958). Additional drugs tested were: D-amphetamine, because of recent reports of psychoses occurring in connection with its use (CONNELL 1958; ASKEVOLD 1959); cocaine, because of the occurrence in addicts of hallucinations and paranoid thinking (GOODMAN and GILMAN 1955); BOL, chlorpromazine, and meprobamate, for purposes of comparison.

Methods

Cholinesterase inhibition was measured by the method of KALOW and GENEST (1957), modified by the substitution of appropriate inhibitors in various concentrations. Determinations of hydrolytic activity, with and without inhibitor, with BzCh (5×10^{-5} M) as substrate, were made by measuring the change in absorbance at 240 mμ in a Beckman DU spectrophotometer equipped with thermospacers. All determinations were made between 25° C and 27° C and the value corrected to 26° C by the formula of KALOW and GENEST (1957). 0.15 M Phosphate buffer at p_H 7.4 was used as solvent throughout. All values reported represent duplicate determinations.

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Whole human blood serum diluted to a final concentration of 1:200 was used as a source of enzyme. The blood with typical cholinesterase was drawn from young adult males free of manifest disease. The blood was allowed to stand at room temperature for 4—6 hours; then it was centrifuged and the serum separated and refrigerated until time of use. Inhibition characteristics remained constant under these circumstances for at least several months.

Atypical enzyme was obtained from patients who had a history of atypical response to succinylcholine. The blood was treated in the same manner as for the typical enzyme.

Except with DFP all inhibitor concentrations indicate the concentration in the final reaction mixture. Among the drugs tested in this study, only DFP required preincubation with the enzyme prior to addition of substrate in order to achieve maximum inhibition. All other drugs were added to the enzyme simultaneously with the substrate. DFP was added to separate samples of diluted serum, both at the time the substrate was added and at measured intervals prior to the addition of substrate. The concentrations of DFP represent the inhibitor in the serum-inhibitor mixture rather than in the final reaction mixture.

For some of the inhibitors the concentration which could be tested effectively in our system was limited by the ultraviolet absorbance of the inhibitor. No attempt was made to test inhibitor concentrations with initial absorbances above 1.000.

Results

Blood samples from two subjects with atypical cholinesterase have been studied in this laboratory. Both were referred because of prolonged apnea after injections of succinylcholine.

Fig. 1 shows the inhibitions obtained with various concentrations of dibucaine at 26° C, p_H 7.4, in M/15 phosphate buffer with BzCh as substrate using the two types of enzymes. Defining the dibucaine number (DN) as the percentage inhibition produced by 10^{-5} M dibucaine under the specified conditions (KALOW and GENEST 1957), the typical enzyme has a DN of 80; the atypical enzyme a DN of 18.

[Serum samples from seven subjects with intermediate dibucaine numbers ranging from 55 to 65 have been examined in this laboratory. Since these sera apparently contain a mixture of typical and atypical enzyme (KALOW and DAVIES 1959) they have not been included in the present study.]

LSD 25. Fig. 2 shows the percentage inhibition obtained with various concentrations of LSD 25 using sera from two subjects with typical cholinesterase and from one subject with atypical cholinesterase.

With LSD 25 as inhibitor the negative logarithm of the molar concentration necessary to produce 50 per cent inhibition (pI_{50}) of typical cholinesterase is 6.55; the pI_0 for atypical cholinesterase is 6.20.

The activation reported to occur with lower concentrations of LSD (FRIED and ANTAPOL 1957) was not observed in this system with concentrations as low as 10^{-10} M.

In addition to the above data, the inhibition produced by a single concentration of LSD 25 ($10^{-6.5}$ M) has been measured individually using serum obtained from 16 young males who were free of manifest disease. All these subjects had typical cholinesterase with DN's ranging from 76 to 83. The per cent inhibition produced by $10^{-6.5}$ M LSD ranged from 49 per cent to 58 per cent (mean 54.8 per cent).

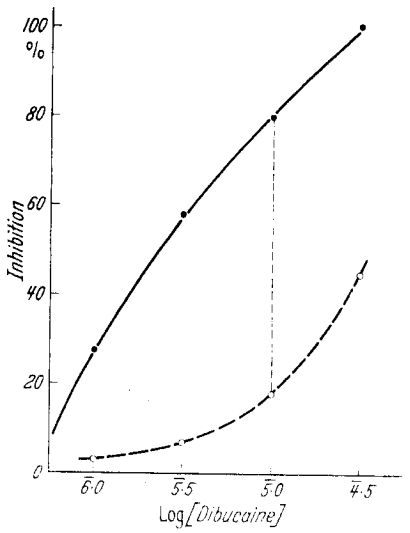

Fig. 1. The inhibition of two types of human serum cholinesterase produced by dibucaine. ●—— Typical; ○- - - -○ Atypical

BOL 148. Fig. 3 shows the percentage inhibition obtained with various concentrations of BOL 148, using sera from one subject with typical cholinesterase and from two patients with atypical cholinesterase. With BOL 148, the pI_{50} for typical cholinesterase is 6.15; the pI_{50} for atypical cholinesterase is 5.70.

Mescaline. Concentrations of mescaline up to 10^{-4} M produced no measurable inhibition of typical cholinesterase. The effect of higher concentrations could not be measured in this system because of the absorbance by mescaline.

N-allylnormorphine. N-allylnormorphine, 10^{-4} M, produced 38 per cent inhibition of typical cholinesterase and six per cent inhibition of atypical cholinesterase. At $10^{-4.5}$ M it inhibited typical cholinesterase 15 per cent but produced no measurable inhibition of atypical cholinesterase. No activation of BzCh hydrolysis was noted at any of the concentrations tested.

Dimethyltryptamine. Inhibition rates for the typical and atypical enzyme, respectively, were 50 per cent and 16 per cent at 10^{-4} M, 25 per cent and 7 per cent at $10^{-4.5}$ M, 11 per cent and 6 per cent at 10^{-5} M, 5 per cent and 4 per cent at $10^{-5.5}$ M, and 0 per cent for both enzymes at 10^{-6} M.

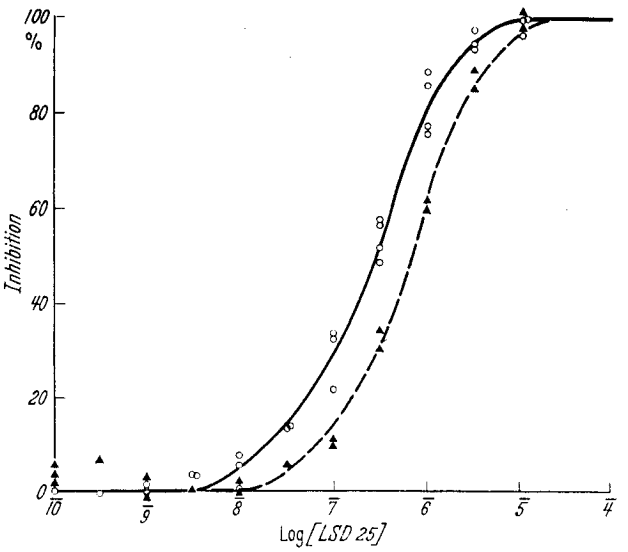

Fig. 2. The inhibition of two types of human serum cholinesterase produced by LSD.
○——○ Typical cholinesterase; ▲——▲ Atypical cholinesterase

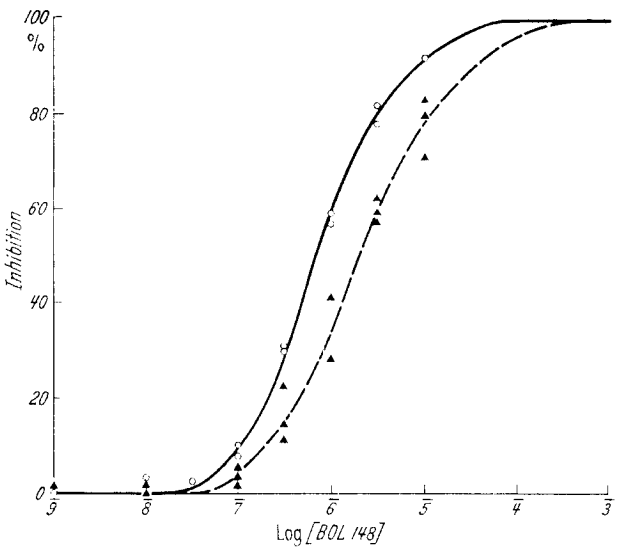

Fig. 3. The inhibition of two types of human serum cholinesterase produced by Brom-LSD.
○——○ Typical cholinesterase; ▲——▲ Atypical cholinesterase

WIN 2299. Typical cholinesterase was inhibited 54 per cent at $10^{-4.5}$ M and 34 per cent at 10^{-5} M. Atypical enzyme was not inhibited measurably at either of these concentrations.

Harmine. Typical cholinesterase was inhibited 9 per cent at 10^{-4} M but not measurably inhibited at $10^{-4.5}$ M. Atypical cholinesterase was not tested.

Cocaine. Cocaine at 10^{-5} M produced 13 per cent inhibition of typical cholinesterase. It was not tested against atypical cholinesterase and could not satisfactorily be tested at higher concentrations due to its absorbance characteristics.

Psilocybin. Typical cholinesterase was inhibited 97 per cent by 10^{-4} M, 68 per cent by $10^{-4.5}$ M, 25 per cent by 10^{-5} M, 5 per cent by

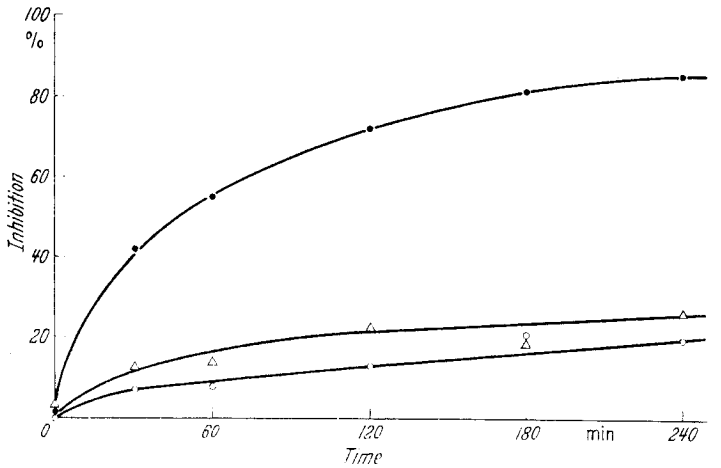

Fig. 4. The effect of preincubation time of serum with inhibitor on the serum cholinesterase inhibition produced by three concentrations of DFP. Concentrations indicate DFP during preincubation rather than in final reaction mixture. ●——● 10^{-8} M DFP; △——△ 2×10^{-9} M DFP; ○——○ 1×10^{-9} M DFP

$10^{-5.5}$ M, and 0 per cent by 10^{-6} M. Atypical cholinesterase was not tested.

Psilocybin solutions became discolored progressively over the first few hours after the sealed ampule was opened, both in the stock solution and in dilute buffered solutions. All the determinations noted above were done within six hours after the ampule was opened. The inhibition produced by 10^{-5} M solution was measured one hour after opening the ampule and five hours after opening the ampule with identical results.

D-*Amphetamine.* D-amphetamine in concentrations up to 10^{-3} M produced no measurable inhibition of typical cholinesterase.

Diisopropylfluorophosphonate (DFP). Fig. 4 shows the effect of DFP on typical cholinesterase with various times of preincubation prior to adding substrate. Addition of DFP to serum simultaneously with substrate resulted in no measurable inhibition. The inhibition observed after preincubation with DFP appears initially to be limited both by the

time of preincubation and by the concentration of the inhibitor, while the degree of inhibition ultimately achieved appears to depend upon the concentration of inhibitor in the enzyme-inhibitor mixture.

Chlorpromazine. Inhibition rates for typical cholinesterase with chlorpromazine were 77 per cent at $10^{-4.5}$ M, 51 per cent at 10^{-5} M, 19 per cent at $10^{-5.5}$ M, 4 per cent at 10^{-6} M, 2 per cent at $10^{-6.5}$ M, and 0 per cent at 10^{-7} M. None of these concentrations produced measurable inhibition of atypical cholinesterase.

Meprobamate. 10^{-4} M meprobamate produced no measurable inhibition of typical cholinesterase.

In Table 1 the drugs tested in this study are tabulated in order of their molar effectiveness as inhibitors of the hydrolysis of BzCh under specified conditions by human serum cholinesterase.

Table 1. *Approximate order of molar effectiveness as inhibitors of typical human serum cholinesterase*

	pI_{50}	pI_{10}
DFP	see Figure 4	
LSD	$10^{-6.5}$	
BOL 148	$10^{-6.0}$	
Chlorpromazine	$10^{-5.0}$	
Psilocybin	$10^{-4.6\pm}$	
WIN 2299	$10^{-4.5\pm}$	
DMT	$10^{-4.0}$	
N-Allylnormorphine	$10^{-3.8}$	
Cocaine		10^{-5}
Harmine		10^{-4}
Meprobamate	No measurable inhibition at 10^{-4} M	
D-Amphetamine	No measurable inhibition at 10^{-3} M	
Mescaline	No measurable inhibition at 10^{-4} M	

Discussion

While a number of drugs which produce psychotomimetic symptoms in man are reasonably potent inhibitors of human serum cholinesterase, this does not appear to be either a necessary or an adequate characteristic for such activity. Thus it appears that inhibition of serum cholinesterase, as evaluated by the system used in this study, is not critically involved in the mode of action of these drugs, at least in so far as they are psychotomimetic.

In support of such a conclusion one notes that BOL, which is not psychotomimetic, is almost as potent a cholinesterase inhibitor as is LSD (see also ZEHNDER and CERLETTI 1956), while chlorpromazine, which is not psychotomimetic and may even antagonize the effects of LSD (HOCH 1955; ISBELL and LOGAN 1957), is itself a cholinesterase inhibitor exceeding in molar effectiveness some of the psychotomimetic drugs. On the other hand, mescaline, a less potent but specific psychotomimetic

agent (KOELLE 1958; BEECHER 1958), has little if any effect on human serum cholinesterase.

To any such generalization, however, certain distinct reservations must be added. One such reservation concerns choice of substrate and assay system. For instance, other investigators using other assay systems (THOMPSON et al. 1955; ZEHNDER and CERLETTI 1956; GOLDENBERG 1956) report 50 per cent inhibition with concentrations of LSD 10 to 20 times as great as that producing 50 per cent inhibition in this assay system (BzCh 5×10^{-5} M; p_H 7.4; PO_4 buffer; 26° C). Similar differences are noted also with BOL and in the GOLDENBERG's study (1956) with chlorpromazine. Since the physiologic substrate of serum cholinesterase is not known, one can only conjecture which, if either, more accurately reflects the inhibition that obtains *in vivo*.

Also, caution is necessary in attempting to extend these observations to the cholinesterase of other tissues, particularly of brain. Although THOMPSON et al. (1955) obtained similar inhibition rates for brain and serum cholinesterase with LSD, as did ORD and THOMPSON (1952) with several other inhibitors, it is still not certain that the enzymes occurring in the two tissues are identical. Several investigators, in fact, have produced evidence that brain contains several hydrolytic enzymes (MYERS 1956; PEPLER and PEARSE 1957) closely resembling "cholinesterase" in substrate specificities but differing somewhat both in specificities for substrates and inhibitors. Accordingly, the data reported here cannot be expected to apply rigidly to the 'cholinesterases' of the nervous system.

In general, our findings have confirmed those of KALOW and his co-workers (KALOW and GENEST 1957; KALOW and STARON 1957) regarding the occurrence of an atypical form of human serum cholinesterase, differing from normal in its sensitivity to a number of inhibitors. Our data show, however, that while the two forms differ in sensitivity to LSD and BOL, the degree of difference does not conform to the mathematical relationship described for inhibitors believed to react with the anionic site of the enzyme (KALOW and DAVIES 1959). Rather, there is a smaller difference than would be anticipated on the basis of an anionic site reaction. On the other hand, DFP and TEPP, believed to react with the esteratic site, affect both enzymes equally (KALOW and DAVIES 1959). This may indicate that LSD and BOL are reacting with both the esteratic and the anionic sites of the enzyme. Whatever the basis for the difference, however, neither LSD nor BOL fits into any of the three classes of inhibitors defined by KALOW and DAVIES (1959).

Summary

The effects of a number of drugs of psychiatric interest on human serum cholinesterase are reported.

The reported occurrence of an atypical form of human serum cholinesterase, differing in sensitivity to some inhibitors, has been confirmed.

LSD and BOL, both potent inhibitors of human serum cholinesterase, do not fit into any of the three groups of inhibitors defined by their relative effectiveness as inhibitors of typical and atypical human serum cholinesterase.

Some of the limitations of these findings are discussed.

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