Biochemical Pharmacology, 1964, Vol. 13, pp. 1359–1361. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

Inability of 4-phenyl-n-butylamine to reverse monoamine oxidase inhibition by tranylcypromine

(*Received* 12 *March* 1964; *accepted* 11 *May* 1964)

ZELLER and Sarkar[1] reported the ability of 4-phenyl-n-butylamine (PBA) to reverse the inhibition by tranylcypromine (*trans*-2-phenylcyclopropylamine) of monoamine oxidase (MAO) activity present in intact and solubilized mitochondria obtained from beef and rabbit liver. However, a closer inspection of the data revealed that a concentration of 1×10^{-6} M (pI_{50}, 6·0) of tranylcypromine was required to inhibit by 50 per cent the oxidative deamination of tyramine by MAO, whereas 400 times as much of the inhibitor (4×10^{-4} M; pI_{50}, 3·4) was necessary to produce the same extent of inhibition of the oxidative deamination of PBA. Accordingly, in the experimental situation of Zeller and Sarkar[1] in which 2×10^{-5} M of the inhibitor was used to inhibit the oxidative deamination of tyramine completely, this concentration of inhibitor was insufficient to block appreciably the oxidative deamination of PBA subsequently added. Therefore, the measured uptake of O_2 under these conditions should not be interpreted as a reactivation of MAO activity, since it undoubtedly did reflect the enzymatic oxidation of the added PBA.

The present study was undertaken to check the ability of PBA to reverse the previously demonstrated inhibition of MAO activity of rat brain by tranylcypromine.[2, 3] The results show that, when assayed directly in terms of the disappearance of substrate (serotonin), the inhibition by tranylcypromine of MAO activity present in rat brain mitochondria was not reversed by PBA. Similar results were obtained with rat liver mitochondrial preparation. In addition, the metabolism of serotonin by MAO was inhibited by the presence of PBA.

EXPERIMENTAL PROCEDURE

Tranylcypromine hydrochloride or sulfate and PBA hydrochloride were used as aqueous solutions. The MAO assay was performed according to the procedure of Sjoerdsma *et al.*[4] and Bogdanski *et al.*[5] by measuring the rate of disappearance of serotonin at pH 7 in phosphate buffer. Serotonin was extracted from the incubation mixture and assayed according to the nitrosonaphthol method of Udenfriend *et al.*[6]

The addition of tranylcypromine or PBA to the incubation was always followed by a 15-min preincubation period. The indicated concentrations represent those present in the incubation mixture.

All incubations were conducted in a Dubnoff metabolic shaker at 37° in the presence of air.

Preparation of brain mitochondria. Male albino rats, Wistar strain, weighing between 100 and 200 g, were sacrificed by decapitation; the brains were immediately removed, rinsed with H_2O to remove excess blood, lightly blotted, and weighed. Several brains were pooled and homogenized at 0° in 4 volumes of 0·25 M sucrose solution. After centrifugation of the homogenate for 15 min at 1,000 *g* at 0°, the supernatant was removed and centrifuged for 30 min at 10,000 *g* in the Spinco model L centrifuge. The sedimented mitochondrial pellet was washed with phosphate buffer (pH 7·0, 0·10 M) and then resuspended in the buffer so that 1 ml of the resulting mixture contained mitochondria equivalent to 300 mg of whole brain. After preincubation for 15 min with PBA or tranylcypromine, the enzyme mixture was centrifuged at 20,000 *g* at 0°. The sedimented residue was washed two or three times with several volumes of phosphate buffer and finally resuspended in the buffer in a volume equal to that of the supernatant removed.

Preparation of rat liver mitochondria. Rat liver mitochondria were prepared and washed in the same manner as described for brain mitochondria.

RESULTS

Rat brain homogenate. Preincubation with PBA inhibited the MAO activity of rat brain homogenate toward serotonin as substrate. The extent of the inhibition increased with increasing concentrations of PBA, as shown in Fig. 1, with 50 per cent inhibition at a concentration of about 2×10^{-4} M.

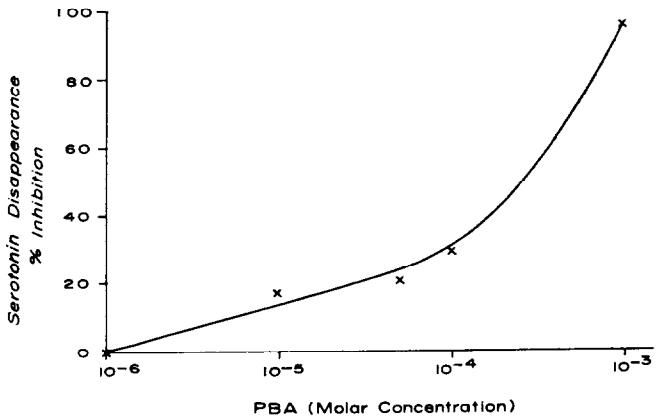

FIG. 1. Effect of phenylbutylamine upon ability of rat brain MAO to metabolize serotonin. A mixture of 1·0 ml of 33% brain homogenate in H_2O, 0·2 ml phosphate buffer (0·5 M, pH 7·0) and 0·8 ml PBA was incubated for 15 min at 37°. After the addition of 1·0 ml of a solution of 300 ug serotonin (final concentration, $5{\cdot}7 \times 10^{4}$ M), the mixture was further incubated for 45 min, and the amount of serotonin that disappeared was measured. In the control H_2O was substituted for PBA.

Rat brain mitochondria. In view of the inhibitory effect of the presence of PBA upon the ability of rat brain MAO to metabolize serotonin, it was necessary to wash the enzyme preparation free from PBA prior to assay of activity. For this purpose a rat brain mitochondrial preparation was used. While preincubation for 15 min at 37° with 5×10^{-4} M and 1×10^{-2} M PBA resulted in inhibitions of MAO activity toward serotonin of 57 per cent and 100 per cent respectively, subsequent washing of the sedimented mitochondria two or three times with phosphate buffer (0·1 M, pH 7·0) restored the MAO activity to normal. The inhibition of MAO activity by tranylcypromine, however, could not be reversed by washing. These results are summarized in Table 1.

TABLE 1. EFFECT UPON MAO ACTIVITY OF WASHING RAT BRAIN MITOCHONDRIA

After preincubation for 15 min with PBA or tranylcypromine, an aliquot of the incubation mixture was assayed for MAO activity and another aliquot was centrfuged at 20,000 *g* for 15 min and the sedimented mitochondria washed with phosphate buffer as described under Experimental Procedure. The washed mitochondria were then assayed for MAO activity.

Pretreatment	Serotonin consumed		Inhibition	
	Before washing	After washing	Before washing	After washing
None	117	117*		
PBA (5×10^{-4} M)	49	112	57	4
PBA (1×10^{-2} M)	0	102	100	12
T* ($2{\cdot}5 \times 10^{-6}$ M)	47	56	60	52
T* (1×10^{-4} M)	5	5	96	96

* Occasionally a small loss (less than 20%) of activity was observed on washing.
† Tranylcypromine.

The optimal concentration of serotonin for maximal MAO activity of brain mitochondria, equivalent to 300 mg of brain tissue, was the same as that found with whole brain homogenate ($5{\cdot}7 \times 10^{-4}$ M). The rate of disappearance of serotonin was proportional to the concentration of enzyme.

Effect of PBA upon MAO activity inhibited by tranylcypromine. Incubation in the presence of 1×10^{-2} M PBA of mitochondrial preparation, whose ability to metabolize serotonin was partially or almost completely inhibited by prior incubation with tranylcypromine, failed to reverse the inhibition by tranylcypromine of serotonin oxidation (Table 2).

TABLE 2. EFFECT OF PBA UPON MAO ACTIVITY OF RAT BRAIN MITOCHONDRIA INHIBITED BY TRANYLCYPROMINE

The addition of each drug to the incubation mixture was followed by a 15-min incubation at 37°. The mitochondria were then sedimented by centrifugation and washed with phosphate buffer as described under Experimental Procedure, prior to enzyme assay.

Pretreatment	Serotonin consumed (μg)	Inhibition %
None	117	
T* (1×10^{-6} M)	94	20
T (1×10^{-6} M), PBA	88	25
T ($2{\cdot}5 \times 10^{-6}$ M)	56	52
T ($2{\cdot}5 \times 10^{-6}$ M), PBA	47	60
T (1×10^{-4} M)	8	93
T (1×10^{-4} M), PBA	2	98

* Tranylcypromine.

Rat liver mitochondria. The MAO activity of rat liver mitochondrial preparation was more than twice that of the brain preparation. Accordingly, half as much of the liver preparation was used. The optimal concentration of serotonin for maximal MAO acitvity was the same as with the brain preparation ($5{\cdot}7 \times 10^{-4}$ M). The rate of disappearance of serotonin was proportional to the concentration of mitochondrial enzyme. As with the brain preparation, incubation in the presence of 1×10^{-2} M PBA of liver mitichondraial preparation, whose ability to metabolize serotonin was partially or almost completely inhibited by prior incubation with tranylcypromine, failed to reverse the inhibition by tranylcypromine of serotonin oxidation.

The results of the present study indicate that the inhibition by tranylcypromine of serotonin metabolism by MAO present in rat brain and liver mitochondria was not reversed by PBA at concentrations to 1×10^{-2} M.

Sarkar and Zeller[7] reported rapid oxidative deamination of PBA by MAO in intact and solubilized rabbit liver mitochondria. The present data show that PBA reversibly inhibited the metabolism of serotonin by MAO and that the degree of inhibition was dependent upon the concentration of PBA in the reaction mixture. Removal of the PBA by washing the mitochondrial preparation with phosphate buffer fully restored the activity of the enzyme toward serotonin.

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