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Sympathomimetic actions of monoamine oxidase inhibitors in the isolating nictitating membrane of the cat*

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VARIOUS monoamine oxidase inhibitors have been found to possess sympathomimetic actions. For example, Goldberg and Shideman,[1] using isolated atria, demonstrated that tranylcypromine exerted a positive inotropic effect which was antagonized by dichloroisoproterenol and prevented by pretreatment of the animals with reserpine. Lee *et al.*[2] made similar observations with several MAO inhibitors in isolated papillary muscles and auricles and further showed that norepinephrine restored the response to the MAO inhibitors in preparations from cats pretreated with reserpine. Ryall[3] reported that pheniprazine caused a marked contraction of the acutely denervated nictitating membrane.

The experiments reported here demonstrate that several MAO inhibitors alter the resting tension of the isolated nictitating membrane and present evidence as to the mechanisms involved. The cats used weighed between 1·5 and 4·0 kg. They were anesthetized with ether and spinal preparations were made as described by Burn.[4] The medial smooth muscle of the nictitating membrane was isolated according to the method of Thompson.[5] The preparation was suspended in a 30-ml bath of Krebs' solution[5] and bubbled with 95% oxygen—5% carbon dioxide. The temperature of the bath was maintained at 37° ± 0·2°. Tension was recorded by means of a Grass strain gauge and polygraph. The tension on the muscle was set at 2 to 3 g by means of a rack and pinion. During the following hour, the Krebs solution in the bath was changed several times. The tension gradually declined during this period, requiring that the tension be readjusted to the 2-3 g initial value. Within 1 hr after placing the muscle in the bath, the tension stabilized and remained constant at 2 to 3 g during the rest of the experiment, except when deliberately altered by the addition of drugs. Tension-response experiments indicated that the optimal tension was between 2 and 4 g.

The addition of tranylcypromine (100 μg/ml), phenelzine (20 μg/ml), pheniprazine (300 μg/ml), pargyline (150 μg/ml), or harmaline (100 μg/ml) to the organ bath caused a contraction of the isolated smooth muscle. Phenelzine was by far the most effective. These results are presented in Table 1 along with a comparison to the effects of norepinephrine (1·0 μg/ml) and tyramine (3·0 μg/ml).

The antagonism of phenoxybenzamine against the contractions produced by norepinephrine and the MAO inhibitors was determined. The response to the agonist was tested and the Krebs solution was changed several times. Phenoxybenzamine (20 μg/ml) was then added to the bath. This resulted in a slow, gradual increase in tension of approximately 1·0 g. Twenty minutes later the Krebs solution was replaced by phenoxybenzamine-free solution. This flushing was repeated two or three times during the next 10 min. The tension returned to the initial value during this time. The response to the agonist was then tested for the second time. Table 1(A) shows that phenoxybenzamine produced marked antagonism against norepinephrine and all five MAO inhibitors. The responses to norepinephrine, pheniprazine, and harmaline were reversed to relaxation in every experiment. In the cases of phenelzine and pargyline, the responses were reversed in two out of three and three out of four experiments respectively. These results indicate that all five MAO inhibitors cause a contraction of the nictitating membrane either by acting directly on the adrenergic receptors or by causing the release of endogenous catecholamines.

Table 1(B) presents data obtained in experiments aimed at distinguishing between these two possibilities. Cats were pretreated with reserpine, 1 mg/kg, i.p., 24 hr prior to the experiment. Previous work by Fleming and Trendelenburg[6] and Kirpekar *et al.*[7] has proven that this dose causes pronounced depletion of the norepinephrine store in the nictitating membrane. Trendelenburg[8] has found that an even smaller dose of reserpine (0·1 mg/kg) considerably reduces the response of the intact mictitating membrane to the indirect acting sympathomimetic, tyramine. The table indicates that such pretreatment with reserpine did greatly reduce the response of the isolated nictitating membrane to tyramine as well as significantly reducing the responses to phenelzine, pheniprazine, and pargyline. On the

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other hand, the responses to tranylcypromine and harmaline were not decreased at all by pretreatment with reserpine. It is suggested that phenelzine, pheniprazine, and pargyline produce a contraction of the nictating membrane by releasing endogenous norepinephrine but that tranylcypromine and harmaline act directly on the adrenergic receptors.

TABLE 1. THE EFFECTS OF PHENOXYBENZAMINE AND OF PRETREATMENT WITH RESERPINE ON THE RESPONSES OF THE ISOLATED NICTITATING MEMBRANE OF THE CAT TO NOREPINEPHRINE, TYRAMINE, AND MONOAMINE OXIDASE INHIBITORS*

Agonist	Dose	A. Contraction (+) or relaxation (−)		P value
		Control	After phenoxybenzamine	
	(μg/ml)	(g)	(g)	
Norepinephrine	1	+ 5·88 ± 0·34 (38)	−1·00 ± 0·10 (20)	<0·001
Tranylcypromine	100	+ 7.57 ± 1·28 (7)	+1·28 ± 0·48 (7)	<0·001
Phenelzine	20	+10·16 ± 2·31 (3)	−0·83 ± 0·14 (3)	<0·01
Pheniprazine	300	+ 6·06 ± 0·93 (4)	−0·43 ± 0·06 (4)	<0·001
Pargyline	150	+ 2·81 ± 0·36 (4)	−1·25 ± 0·22 (4)	<0·001
Harmaline	100	+ 1·81 ± 0·61 (4)	−2·31 ± 0·43 (4)	<0·005
		B. Contraction		
		Control	Reserpine	
Tyramine	3	9·13 ± 0·33 (20)	2·01 ± 0·42 (19)	<0·001
Tranylcypromine	100	7·57 ± 1·28 (7)	8·86 ± 0·50 (4)	>0·4
Phenelzine	20	10·16 ± 2·30 (3)	4·31 ± 0·30 (4)	<0·05
Pheniprazine	300	6·06 ± 0·93 (4)	2·75 ± 0·87 (4)	<0·05
Pargyline	150	2·81 ± 0·36 (4)	1·35 ± 0·20 (4)	<0·025
Harmaline	100	1·81 ± 0·62 (4)	3·12 ± 0·52 (4)	>0·1

* To test the antagonism of phenoxybenzamine, the response to an agonist was determined in a preparation before and again after the addition of phenoxybenzamine (20 μg/ml) to the bath. To test the effect of reserpine, the responses of membranes taken from cats pretreated with reserpine (1·0 mg/kg) 24 hr before the experiemnt were compared to the responses of control preparations. The numbers in parenthesis indicate the number of preparations tested. Values are means ± standard error.

Unlike those MAO inhibitors discussed above, iproniazid (300 and 500 μg/ml) caused a relaxation of the isolated nictitating membrane. It was considered possible that the iproniazid was acting on β adrenergic receptors. Smith[9] has presented evidence that in the nictitating membrane there are β receptors which lead to relaxation. However, dichloroisoproterenol (10 μg/ml) did not block either the relaxation produced by iproniazid or the relaxation produced by pargyline and harmaline in the presence of phenoxybenzamine. Since this dose of dichloroisoproterenol causes marked antagonism of the inhibitory effect of isoproterenol in the isolated nictitating membrane, the relaxation produced by MAO inhibitors in this preparation must not be through the action of β receptors.

There are apparently species and/or tissue differences in regard to the mechanisms by which particular MAO inhibitors produce sympathomimetic effects. The failure of depletion of norepinephrine stores in the nictitating membrane to prevent the stimulating action of tranylcypromine is in contrast to the finding of Goldberg and Shideman[1] that the positive inotropic action of tranylcypromine on isolated cat atria was prevented by pretreatment of the animals with reserpine. Pepeu *et al.*[10] found that the positive inotropic and chronotropic effects if iproniazid observed in isolated guinea pig atria were blocked by dichloroisoproterenol, whereas we observed that the inhibitory effect of iproniazid in the isolated nictitating membrane was not antagonized by the β blocker. It is quite possible that the inhibitory effect of iproniazid in the nictitating membrane is not sympathomimetic in nature.

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Characterization of the glucuronide conjugate of chlorphenesin carbamate from the rat and from man

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THE excretion and metabolism of chlorphenesin carbamate* [1,2-propanediol,3(*p*-chlorophenoxy)-1-carbamate] in rats and humans has recently been described.[1] A major portion of the drug was excreted into the urine as a conjugate which could be hydrolyzed by incubation with β-glucuronidase. The recent characterization of the N-glucuronide derivatives of the carbamate-type drugs meprobamate[2, 3] and ethinamate,[4] however, suggested the possibility that chlorphenesin carbamate might be excreted as both a carbamate N-glucuronide as well as the conventional O-glucuronide. Therefore, it was of considerable interest to establish the nature of the chlorphenesin carbamate conjugates excreted by both the rat and by man.

METHODS

Normal human subjects received 2 g of (±)-chlorphenesin carbamate orally in capsules, and urine was collected over a 24-hr period. In animal studies, four male Wistar rats were each given oral doses of 200 mg (±)-chlorphenesin carbamate/kg every 24 hr for 5 days. The excreted urine was collected for 6 days and pooled. The crude glucuronide fraction was isolated from the urine samples by the basic lead acetate precipitation method of Kamil *et al.*[5] The resultant glucuronide gum was methylated with ethereal diazomethane and then acetylated with acetic anhydride in the presence of pyridine. The crude glucuronide ester mixtures were purified by chromatography on either a silica gel column with ethyl ether–benzene and ethyl ether–ethyl acetate mixtures of increasing polarity or on

* Registered by The Upjohn Company under the trade name of Maolate.