THE ROLE OF ADRENERGIC MECHANISM IN TREMORINE-INDUCED TREMORS IN RATS: ANTITREMOR EFFECT OF β-ADRENOCEPTOR ANTAGONISTS

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Summary : Tranylcypromine (TCP) pretreatmant was found to accelerate the tremorogenic activity of tremorine in rats. Conversely, reserpinization delayed the onset of induction of tremors, and a significant diminution in their intensity was observed in these rats. A comparative study of the antitremor activity of β-adrenoceptor antagonists against this tremor-model showed that butoxamine (β_2-antagonist) and propranolol (nonselective antagonist) were able to afford a rapid and powerful protection, whereas a weaker and delayed effect was observed in rats treated with the β_1-antagonist, acebutolol, Furthermore, the antitremor activity of butoxamine and propranolol but not that of acebutolol was found of be potentiated and diminished in rats pretreated with reserpine and TCP, respectively. It was inferred that β_2-receptor modulated the tremorogenic activity of tremorine, and that inhibition by propranolol or butoxamine of this subtype β-adrenoceptor resulted in rapid and powerful suppression of tremors, and that the antiadrenergic activity of acebutolol was unlikely to have a role in its antitremor effect.

Key words : Acebutolol butoxamine propranolol tremorine-induced tremors

INTRODUCTION

Since atropine but not its quaternary substitutes inhibited both tremors and the peripheral action of tremorine, induction of tremors has been suggested to involve centrally located muscarinic receptors (1). Inhibition by adrenergic β-receptor antagonists of tremors but not the peripheral actions (12, 13) suggests that an adrenergic mechanism participates in the central muscarinic action of tremorine. To obtain further information on the role of adrenergic mechanism in genesis of tremors, the tremorogenic potential of tremorine was tested in this study following functional alteration of adrenergic mechanism in rats with a monoamine-oxidase inhibitor, tranylcypromine (TCP) or a noradrenaline (NA) depletor, reserpine. In another set of experiment, the activity of nonselective β-adrenoceptor antagonist, propranolol was compared with that of acebutolol or butoxamine, the blocking activity of which has been relatively specific on β_1 (11) or β_2-adrenocep-

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tors (8), respectively, in order to evaluate their antitremor effect. The action of these drugs was tested in TCP or reserpine pretreated rats, to elucidate whether blockade of adrenergic β-receptor populations or a different mechanism in involved in their antitremor action.

MATERIAL AND METHODS

Albino rats (Swiss) of either sex weighing 150-200 *g* were used. All drugs were freshly prepared in sterile distilled water and injected (0.2 *ml*/100 *g*). Tremorine (10 *mg/kg*; Sigma, U.S.A.) was injected in groups of reserpine (5 *mg/kg*, 24 hr before) or TCP 10 *mg/kg* 1 hr before) pretreated rats. In another group of rats, acebutolol (May & Baker), butoxamine (Burroughs Wellcome) or propranolol (Cipla, India) was injected 30 min prior to tremorine challenge. To ascertain the latency to their antitremor activity, rats were dosed with test drugs 10 min after tremorine treatment yet another group of reserpine or TCP pretreated rats received, 30 min before tremorine challenge a dose of one of the test drugs. All control animals received equivalent volume of the vehicle.

Following tremorine treatment, rats were caged singly for observation. The latency (min to the appearence of frist tremor) and the intensity of tremors were recorded in each rat. A 0-4 scale scoring method (13) was used to assess the severity of tremors: (no tremors = 0, intermittent mild tremors = 1, persistent mild tremors = 2, moderate tremors = 3, severe tremors = 4. Student's t test and Mann Whitney rank order test were used for analysis of data on latency and tremor scores, respectively.

RESULTS

After a latent period of 6-8 min, tremorine injected control rats showed increased salivation and lacrimation, and 2-3 min later tremors appeared. The intensity of tremors gradually increased and a peak effect was scored at 20-30 min. A dose-dependent protection was observed in acebutolol, butoxamine and propranolol pretreated rats. The equipotent dose of acebutolol was, however noticeably larger than that of other two drugs (Table I-A). Butoxamine and propranolol were able to inhibit tremors when injected 10 min after tremorine, but acebutolol failed to protect these rats (Table I-B),

The tremorogenic potential but not the peripheral actions of tremorine was found to be significantly altered in reserpine and TCP pretreated rats. Results presented in Table 2 show that in reserpinized rats the onset of tremors was significantly delayed and tremor intensity was decreased. A mild but significant potentiation of the effect of butoxamine and propranolol was observed in these rats. TCP pretreatment, on the other hand, shortened the latency to tremors. A significant reduction in the protective effect of butoxamine and propranolol was observed in these rats. Changes by reserpine or TCP of adrenergic activity, however failed to affect the antitremor property of acebutolol.

TABLE I : Effect of β-adrenoceptor antagonists on tremors when injected beforc (A) or after (B) tremorine.

	Drug and dose (mg/kg, ip)		*Onset (min)*	*Score 30 min after tremorine mean±S.E.M.*
(A)	Distilled water		9.8±1.6	3.6±0.2
	Acebutolol	4	8.2±1.6	3.4±0.2
		8	12.6±1.2	2.8±0.4+
		16	16.8±2.6*	2.1±0.2+
	Propranolol	2	16.5±2.1*	3.0±0.3
		4	20.8±3.2*	2.6±0.2+
		8	27.2±4.8**	1.8±0.2++
	Butoxamine	2	11.8±1.6	3.2±0.3
		4	17.6±3.4*	2.8±0.2+
		8	21.5±4.2*	2.2±0.2++
(B)	Distilled water			3.4±0.2
	Acebutolol	8		3.2±0.3
		16		3.0±0.2
	Propranolol	4		2.6±0.2*
		8		2.0±0.1**
	Butoxamine	4		2.8±0.2*
		8		2.2±0.1**

Rats (10 in each group) received test drugs 30 min before (A) or 10 min after (B) 10 *mg/kg* of tremorine. Latency data are mean ± S.E.M. of rats that responded.

(A) * P<0.05, ** P< 0.01 (Student's t test)
+ P<0.05, ++ P<0.01 (Mann Whitney rank order test).

(B) * P<0.05 ** P<0.01 (Mann Whitney rank order test).

DISCUSSION

Modulation of central muscarinic receptor activity by adrenergic mechanism has previously been suggested since endogenously released NA facilitated excitatory responses of iontophoretically applied acetylcholine on cortical neurons (14,15). Consistently, the present data show potentiation (acceleration of onset of tremors) of the activity of tremorine in TCP pretreated rats. In reserpinized rats, as expected, a prolongation of latency to tremors and a significant diminution of tremor intensity were noted (Table II-A). These findings together with the data indicating that there is potentiation or inhibition of the activity of propranolol but not that of acebutolol in rats pretreated with reserpine (Table II-A) or TCP (Table II-B) respectively, suggest that adrenergic β_2-receptor mechanism is likely to modulate the central muscarinic activity of tremorine.

TABLE II : Effect of reserpine or TCP pretreatment on the action of tremorine and on the antitremor activity of β-adrenoceptor antagonists.

	Pretreatment	*Drug and dose (mg/kg, ip)*	*Onset (min)*	*Score 30 min after tremorine mean ± S.E.M.*
(A)	Distilled water	—	8.3±1.6	3.8±0.2
	Reserpine	—	17.4±2.2*	3.0±0.1+
	Distilled water	Acebutolol - 8	14.5±1.2	2.5±0.1
	Reserpine	Acebutolol - 8	15.8±2.4	2.1±0.1
	Distiilled water	Propranolol - 4	21.2±3.6	2.1±0.1
	Reserpine	Propranolol - 4	27.8±2.4*	1.0±0.3+
	Distilled water	Butoxamine - 4	18.2±2.4	2.2±0.2
	Reserpine	Butoxamine - 4	28r6±4.2*	1.4±0.2+
(B)	Distilled water	—	9.2±1.8	3.6±0.2
	TCP	—	5.1±0.8*	3.8±0.2
	Distilled water	Acebutolol - 8	13.8±2.1	2.6±0,3
	TCP	Acebutotol - 8	12.4±1.2	2.4±0.2
	Distilled water	Propranolol - 4	18.6±3.4	2.2±0.1
	TCP	Propranolol - 4	13.8±1.0*	3.0±0.2+
	Distilled water	Butoxamine - 4	17.6±3.1	2.3±0.3
	TCP	Butoxamine - 4	11,8±1.2*	3.2±0.4+

Twentyfour hr after 5 *mg/kg* reserpine (A) or 1 hr after 10 *mg/kg* of TCP (B) treatment groups (10 in each) of rats were injected with tremorine (10 *mg/kg*). Similarly pretreated rats received test drugs 30 min before tremorine challenge. Latency data are mean±S.E.M. of rats that responded.

* $P<0.05$ (Student's t test), +$P<0.05$ (Mann Whitney rank order test) compared to the respective control group.

Although, at higher dose levels acebutolol is known to block β-receptors nonselectively (11), a rapid protective effect like that of propranolol has not been obtained here even with 16 *mg/kg* of acebutolol. This finding together with the results showing that there is no alteration of its antitremor activity in reserpinized or TCP pretreated rats, suggests that inhibition by acebutolol of tremors has not resulted from blockade of adrenergic β-receptor populations. Its effectiveness may be attributed to its membrane stabilizing property (9), as acebutolol at presently used dose levels is likely to produce this action. The data previously obtained with metoprolol, which exerts no membrane stabilizing action (3) against oxotremorine-induced tremors (5), however do not agree with this hypothesis. Results presented in Table I-A clearly indicate that acebutolol is effective, provided that it is administered prior to tremorine. These data indicate a delayed action of acebutolol, as was shown previously by the β_1-antagonist, metoprolol (9) against tremors induced by the biometabolite of tremorine, oxotremorine (5). It is assumed,

although an appropriate information is lacking in the literature, that these compounds may have facilitated inhibitory synaptic transmission, resulting in prevention of induction of tremors.

There is no direct evidence that blockade of centrally located β_2-adrenoceptors brings about inhibition of tremors, although the β_2-selective blocker, ICI 118 551 (2) has previously been shown to inhibit motor response induced by oxotremorine (5). A central mechanism is likely, provided that enough of butoxamine enters the brain possibility of which is yet to be established in experimental animals. However its effectiveness against electroshock convulsions (6) and in the present tremor-model indicate crossing by butoxamine of the blood-brain barrier in a significant quantity.

The curare-like action (4,7) and inhibition of isoprenaline-induced tremors by propranolol (10) have been attributed to inhibition of adrenergic β_2-receptor activity in the skeletal muscles. If it is a likely explanation for suppression of tremors also, then the rapid and powerful protection that was achieved with propranolol and butoxamine may also be partly explained on the basis of peripheral mechanism.

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REFERENCES

1. Bebbington, A. and R. W. Brimblecombe. Muscarinic receptors in the peripheral and central nervous system. *Adv. Drug Res.*, **2** : 143-172, 1965.

2. Bilski A., S. Dorries, J.D. Fitzerald, R. Jessup, H. Tucker and J. Wale. ICI 118 551 a potent β_2-adrenoceptor antagonist, *Br. J. Pharmac.*, **69** : 292 P. 1980.

3. Brogden, R.N., R.C. Heel, T.M. Speight and G.S. Avery. Metoprolol : a review of its pharmacolgical properties and therapeutic efficacy in hypertension. *Drugs*, **14** : 321-348, 1977.

4. Chiarandini, D.J. Curare-like effect of propranolol on rat extraocular muscles. *Br. J. Pharmac.*, **69** : 13-19, 1980.

5. Hallberg. H. and O. Almgren. Possible involvement of both central and peripheral β-adrenoceptor in tremor mechanism. *Acta. Physiol. Scandinavica (Supplementum)*. **508** : 47, 1982.

6. Iyer. K.S. and R.V.A. Thampuran. β-blockade and anticonvulsant activity of propranolol. *Ind J. Physiol. Pharmac.*, **22** : 293-295, 1978.

7. Larsen, G. and H. Teravanien. Effects of the optical isomers of propranolol on neuromusular transmission in the rat. *Exp. Neurol.*, **59** : 435-443 1978.

8. Levy, B. The adrenergic blocking activity of N-tertbutyl methoxamine (butoxamine). *J. Pharmac. Exp. Ther.*, **151** : 413-422, 1966.

9. Marmo, E., G. Spaziante, D. Pentimalli, A. Marfella, E. Ruggiero, R. Decarla, A. Ruta and G. Matera. Specific and nonspecific activities of acebutolol, atenolol metoprolol and propranolol. Comp arative experimental studies, *Int. J. Clin. Pharmac. Res.*, **2** : 41-48, 1982.

10. Perucca. E., H. Pickler, R. Quy and A. Richens. A. standardized method of assessing the effect of β-adrenoceptor blocking drugs upon isoprenaline-induced physiological tremor and tachycardia : a test for cardioselectivity. *Br. J. Pharmac.*, **72** : 133 P, 1981.

11. Prichard, B.N.C. β-adrenergic receptor blocker in hypertension, past, present and future. *Br. J. Clin. Pharmac.*, **5** : 379-399, 1978.

12. Seth, S.D., G. Jagadesh and B.P. Srinivasan. Antitremorine effect af some β-adrenoceptor blocking drugs in mice - a preliminary communication. *Ind. J. Med. Res.*, **67** : 325-328, 1978.

13. Sharma, P.L. Mechanism of antitremorine activity of adrenergic beta-receptor antagonists in the rat. *Quat. J. Exp. Physiol.*, **55** : 202-206, 1970.

14. Waterhouse, B.D., H.C. Moises and D.J. Woodward. Noradrenergic modulation of somotosensory cortical neuronal responses to iontophoretically applied putative neurotransmitters. *Exp. Neurol.* **69** : 30-49, 1980.

15. Waterhouse, B.D. and D.J. Woodward. Interaction of norepinephrine with cerebrocortical activity evoked by stimulation of somotosensory afferent pathways. *Exp. Neurol.*, **67** : 11-34, 1980.