J. Pharm. Pharmac., 1976, **28**, 286–289 *Received June* 2, 1975

Tremorine-oxotremorine-induced tremor, hypothermia and analgesia, and physostigmine toxicity, in mice after pretreatment with β-adrenoceptor antagonists

F. S. K. BARAR* AND B. R. MADAN**

Department of Pharmacology, S.M.S. Medical College, Jaipur-302004, Rajasthan and ***Department of Pharmacology, S.P. Medical College, Bikaner-334001, Rajasthan, India*

The β-adrenoceptor blockers propranolol, PhQA33 and LB-46 exhibited appreciable activity against tremorine-(TMN) and oxotremorine-(OTMN) induced tremor, whereas pronethalol, (+)-H56/28, (−)-H56/28, Kö-592 and L(+)-INPEA possessed weak action. The two β-blockers, namely D,L(±)-INPEA and D(−)-INPEA acted as weak tremorgens. None of the above compounds suppressed the induced peripheral cholinergic phenomena; or possessed any central anticholinergic activity, as they were unable to afford protection against physostigmine-induced death. Propranolol, PhQA33 and LB-46 antagonized TMN-induced hypothermia and analgesia, but were inactive against OTMN-induced changes. A correlation of the β-blocking and anti-tremor activity of these agents is unlikely.

Herring (1964) reported that pronethalol reduced parkinsonian tremor in man. However, in another clinical study results obtained with propranolol were discouraging (Vas, 1966). In experimental animals, propranolol and practolol were found to antagonize drug-induced tremors (Agarwal & Bose, 1967; Achari & Sinha, 1967, 1968; Cox & Potkonjak, 1970). Further, pronethalol and propranolol attenuated the increase in the amplitude of physiological tremors induced by adrenaline and isoprenaline (Owen & Marsden, 1965; Marsden, Foley & others, 1967).

Ten β-adrenoceptor blocking agents have been selected for elucidation of their antiparkinsonian profile. Preliminary screening was by testing the ability of the drugs to antagonize tremorine-(TMN)-induced tremor (Everett, 1956). Those found effective were then further investigated for their ability to antagonize oxotremorine-(OTMN)-induced tremor, hypothermia and analgesia, and death induced by physostigmine.

MATERIALS AND METHODS

Drugs were administered to groups of male albino mice (Haffkine strain), 20 to 30 g, (n = 6 or 5) in graded doses of 14, 20, 28, 40 and 56 mg kg^{-1} (i.p.) in a saline volume of 0·1 ml per 10 g body weight. Control groups received matching volumes of saline. The mice had free access to food. Twenty min later TMN (20 mg kg^{-1}, s.c.—the dose found by Spencer, 1965, to be most suited as it induced maximal tremor and hypothermia coincided) or OTMN (0·5 mg kg^{-1}, i.p.—a submaximal dose found by Cox & Potkonjak, 1970, to be well suited as tremor onset was almost immediate) or physostigmine (2 mg kg^{-1}, i.p.—a dose found by Nose & Kojima, 1970, to induce 100% mortality within 20 min) was administered, followed by the respective procedure. The ED50 ± s.e. was computed by the method of Miller & Tainter (1944).

Antagonism of TMN (20 mg kg^{-1}, s.c.)—OTMN (0·5 mg kg^{-1}, i.p.) tremors

The method of Funcke, Mulder & others (1969) was used. Tremor recording was started immediately after tremorgen administration. Tremor intensity was recorded 8 times (over 80 min), by an observer, unaware of treatment, using the scoring system: 0 = no tremor. 1 = hardly any tremor. 2 = moderate tremor. 3 = heavy tremor.

The maximum score for a group (n = 6) was 6 × 8 × 3 = 144. Control groups did not attain more than 74% of the maximum. The ED50 was the dose causing 50% reduction of the total tremor score. Changes in cholinergic phenomena like lacrimation, salivation and defaecation were also noted.

Antagonism of TMN (20 mg kg^{-1}, s.c.)—OTMN (0·5 mg kg^{-1}, i.p.) hypothermia and analgesia

The technique employed by Hermansen (1968) was adopted in essential details. Drug-treated groups (n = 6) received TMN–OTMN as above. The

*Correspondence

rectal temperature was measured by an electronic thermometer. The hypothermic effect was followed in each animal by three half hourly determinations, and the lowest reading was used for final calculations. Analgesia was measured by applying an artery clip at the root of the tail according to Haffner's method as cited by Chen (1958). If the mouse did not bite the clip within 15 s of its application, the test for analgesia was recorded as positive (6/6 complete analgesia; 0/6 no analgesia). Statistical significance was assessed by Student's *t*-test ($P < 0.05$).

Antagonism of physostigmine (2 mg kg^{-1}, i.p.) death

The method of Nose & Kojima (1970) was employed. Drug-treated groups (n = 5) received physostigmine as above. The number of mice which survived more than 60 min was recorded for each group, and the ED50 was the dose saving 50% of animals.

The drugs used were: (±)-propranolol HCl; (±)-pronethalol HCl; (+)-1-(2-allylphenoxy)-3-isopropylaminopropanol(−2) HCl ((+)-H56/28); (−1)-1-(2-allylphenoxy-3-isopropylaminopropanol-(−2) bitartrate monohydrate ((−)-H56/28); (±)-1-(3-methylphenoxy)-2-hydroxy-3-isopropylaminopropane HCl (Kö-592); (±)-1-(isopropylamino)-3-(*o*-phenoxyphenoxy)-2-propanol HCl $\frac{1}{2}H_2O$ (PhQA33); (±)-4-(2-hydroxy-3-isopropylaminopropoxy)-indole (LB-46); DL(±)-1-(4-nitrophenyl)-2-isopropylaminoethanol HCl (DL(±)INPEA); D(−)-1-(4-nitrophenyl)-2-isopropylaminoethanol HCl (D(−)-INPEA); L(+)-1-(4-nitrophenyl)-2-isopropylaminoethanol HCl (L(+)-INPEA); tremorine dihydrochloride and oxotremorine sesquifumarate (Aldrich Chemical Co., U.S.A.); physostigmine sulphate (Macfarlan Smith, Edinburgh) and atropine sulphate (Boehringer) were used as standards.

All the compounds were dissolved in 0·9% saline at the required concentration, except LB-46 which was dissolved in 0·9% saline containing an equimolar quantity of tartaric acid. All doses refer to the salts, except LB-46 which was used as a base.

RESULTS

Out of the ten compounds only propranolol, PhQA33 and LB-46 possessed appreciable activity against TMN-tremor, with their ED50 ± s.e. as 15·49 ± 3·5, 23·44 ± 4·1 and 19·05 ± 2·8 mg kg^{-1} (i.p.) respectively, whereas pronethalol, (+)-H56/28, (−)-H56/28, Kö-592 and L(+)-INPEA in high doses of 56 mg kg^{-1} (i.p.) induced a reduction of only 7·4, 38·2, 36·3, 24·7 and 7·8% respectively. With pronethalol TMN-tremor antagonism showed an upward trend up to 28 mg kg^{-1} (25·9%), and a decline at the higher dose-step of 56 mg kg^{-1} (7·4%). DL(±)-INPEA and D(−)-INPEA on the contrary in a dose of 56 mg kg^{-1} (i.p.) increased the tremor score by 8·4 and 9·1% respectively.

The compounds propranolol, PhQA33 and LB-46 also antagonized OTMN-tremor, although at a higher dose level, their ED50 ± s.e. were 26·30 ± 6·8, 30·90 ± 6·9 and 23·44 ± 6·1 mg kg^{-1} (i.p.) respectively. However, none of these three compounds offered any protection against physostigmine-induced death (see Table 1). They (propranolol, PhQA33, LB-46) exerted no influence on the TMN-, OTMN- and physostigmine-induced cholinergic phenomena. Atropine antagonized TMN-, OTMN-tremor and physostigmine-death with its ED50 ± s.e. as 7·24 ± 0·69, 9·33 ± 1·26 and 9·55 ± 2·90 mg kg^{-1} (i.p.) respectively (see Table 1). It also antagonized the associated cholinergic phenomena.

Table 1. *The effect of propranolol, PhQA33, LB-46 and atropine on tremorine-oxotremorine-induced tremor, and physostigmine-induced death in mice.*

	Antitremor activity (n = 6)		
Compound	*vs* Tremorine (20 mg kg^{-1}, s.c.) ED50 ± s.e.*	*vs* Oxotremorine (0·5 mg kg^{-1}, i.p.) ED50 ± s.e.	*vs* Physostigmine death (n = 5) (2 mg kg^{-1}, i.p.) ED50 ± s.e.
Propranolol	15·49 ± 3·5	26·30 ± 6·8	N.P.**
PhQA33	23·44 ± 4·1	30·90 ± 6·9	N.P.
LB-46	19·05 ± 2·8	23·44 ± 6·1	N.P.
Atropine	7·24 ± 0·69	9·33 ± 1·26	9·55 ± 2·90

* mg kg^{-1}, i.p., ** No protection.

The mean rectal temperature of 50 saline-treated mice was 36·2 ± 0·07°. After administration of TMN and OTMN a significant ($P < 0.001$) hypothermia was registered, the rectal temperature being 33·5 ± 0·08 and 33·6 ± 0·09° respectively. Simultaneously TMN caused analgesia in 48 of 50 (48/50) mice and OTMN in 47 of 50 (47/50) mice, while all saline-treated mice responded to the pain stimulus (see Table 2). Propranolol on its own in a dose of 56 mg kg^{-1} (i.p.) significantly ($P < 0.05$) lowered the rectal temperature from 36·2 to 35·2°, while PhQA33 and LB-46 exerted an insignificant ($P > 0.1$) effect (column 3). In a dose of 56 mg kg^{-1} (i.p.) propranolol, PhQA33 and LB-46 exhibited appreciable antagonism of TMN-induced hypothermia ($P < 0.001$) and analgesia (columns 4 & 5), although they were inactive against OTMN (columns 6 & 7). Propranolol in contrast exaggerated OTMN-induced hypothermia ($P < 0.01$). Atropine in a dose of 5 mg kg^{-1} (i.p.) (even lower than its anti-TMN-OTMN-tremor ED50) antagonized TMN-OTMN-induced hypothermia ($P < 0.001$) and

Table 2. *Effect of propranolol, PhQA33, LB-46, and atropine on tremorine and oxotremorine-induced hypothermia and analgesia in mice. Room temp: 29°.*

Compound	Dose mg kg^{-1} i.p.	Saline Rectal temp°C*	TMN (20 mg kg^{-1}, s.c.) Rectal temp °C	TMN Analgesia**	OTMN (0·5 mg kg^{-1}, i.p.) Rectal temp °C	OTMN Analgesia
Saline		36·2 ± 0·07 (50)	33·5 ± 0·08 (50)	48/50	33·6 ± 0·09 (50)	47/50
Propranolol	14	35·9 ± 0·2	34·8 ± 0·4	4/6	33·4 ± 0·4	6/6
	20	35·7 ± 0·2	34·9 ± 0·3	4/6	33·7 ± 0·3	6/6
	28	35·7 ± 0·4	35·7 ± 0·2	4/6	33·7 ± 0·4	6/6
	40	35·4 ± 0·2	35·6 ± 0·2	3/6	32·8 ± 0·5	6/6
	56	35·2 ± 0·4	35·9 ± 0·3	0/6	32·6 ± 0·3	6/6
PhQA33	14	36·6 ± 0·2	33·8 ± 0·3	6/6	33·4 ± 0·3	6/6
	20	36·6 ± 0·2	34·0 ± 0·2	5/6	34·1 ± 0·4	6/6
	28	36·4 ± 0·4	34·6 ± 0·4	4/6	34·1 ± 0·2	6/6
	40	36·1 ± 0·3	34·6 ± 0·2	2/6	33·7 ± 0·4	5/6
	56	35·9 ± 0·5	35·1 ± 0·4	2/6	33·6 ± 0·5	6/6
LB-46	14	36·5 ± 0·2	34·8 ± 0·4	5/6	34·6 ± 0·3	6/6
	20	36·4 ± 0·2	34·6 ± 0·3	4/6	33·6 ± 0·4	6/6
	28	35·8 ± 0·3	35·2 ± 0·4	4/6	34·3 ± 0·2	6/6
	40	35·9 ± 0·5	35·5 ± 0·5	2/6	34·1 ± 0·4	6/6
	56	36·1 ± 0·4	35·7 ± 0·2	1/6	33·7 ± 0·4	5/6
Atropine	5	36·1 ± 0·4	36·1 ± 0·4	4/6	36·3 ± 0·3	1/6
	7	35·9 ± 0·2	36·3 ± 0·3	0/6	35·9 ± 0·4	0/6
	10	36·8 ± 0·4	36·2 ± 0·3	0/6	35·7 ± 0·2	0/6
	14	36·5 ± 0·3	36·4 ± 0·4	0/6	35·9 ± 0·3	0/6
	20	36·9 ± 0·4	36·4 ± 0·2	0/6	36·4 ± 0·2	0/6

* Mean ± s.e. (n = 6, when not indicated in parentheses). ** 6/6 ≡ 100% analgesia, 0/6 ≡ no analgesia.

analgesia. With atropine (20 mg kg^{-1}, i.p.) alone an insignificant ($P > 0.05$) rise in rectal temperature from 36·2 to 36·9° was observed (see Table 2).

DISCUSSION

In the present study propranolol, PhQA33 and LB-46 exhibited significant antagonism of tremor induced by TMN and OTMN, while pronethalol, (+)-H56/28, (−)-H56/28, Kö-592 and L(+)-INPEA had weak action. In contrast DL(±)-INPEA and D(−)-INPEA increased the TMN-tremor score. β-Adrenergic sympathomimetics are known to aggravate parkinsonian tremor (Constas, 1962; Marsden & Owen, 1966; Marshall & Schneiden, 1966). This effect could be blocked by propranolol but not by phentolamine (Constas, 1962; Owen & Marsden, 1965). Moreover, noradrenaline, a predominant α-agonist did not intensify the tremor of parkinsonian disease. Thus, accumulated evidence indicates that the β-adrenoceptors, the presence of which in the brain has been suggested (Bartolini & Pepeu, 1970), are in some way involved in parkinsonian tremor. In corroboration of the above Sharma (1970) and Sharma, Singh & Dhawan (1971) have documented a correlation between β-blocking and anti-TMN activity of (±)-propranolol and (±)-INPEA. However, our results from TMN-tremor studies are not in line with the above viewpoint as reasoned below: (i) (+)-H56/28 and (−)-H56/28 have nearly equal antitremor activity, but as a β-blocker the (+)-form is only 1/100th as potent as the (−)-form (Åblad, Brogård & Ek, 1967), (ii) LB-46 which is 10 times stronger than propranolol as a β-blocker (Giudicelli, Schmitt & Boissier, 1969) has nearly the same antitremor activity as the latter, and (iii) D(−)-INPEA, which is a specific β-blocker (Hahn, Pendleton & Wardell, 1968), intensifies rather than mitigates TMN-tremor. Thus we have failed to support a relationship between the β-blocking and anti-tremor activity.

TMN and OTMN, both direct central muscarinic tremogens, elevate brain acetylcholine concentrations, and this elevation is antagonized by centrally active antiacetylcholine drugs (Cox & Potkonjak, 1969a,b, 1970; Campbell, Hanin & Jenden, 1970). Hence, agents which lower the brain acetylcholine concentrations can be expected to antagonize TMN- and OTMN-tremor. With relevance to this Khanna & Madan (1973a,b) found that the total brain acetylcholine content was significantly decreased by propranolol, (+)-H56/28, (−)-H56/28, Kö-592, PhQA33 and LB-46, while the INPEA's significantly elevated it. These findings are in general agreement with the anti-tremor profile seen in this study,

excepting D(−)-INPEA which oddly showed weak anti-tremor activity. Thus an involvement of central cholinergic mechanism(s) in the antitremor activity of β-blockers is possible. Propranolol, PhQA33 and LB-46 did not suppress the signs of induced cholinergic overactivity in doses which offered protection against TMN- and OTMN-tremor, whereas atropine effectively did so, indicating an absence of a significant peripheral atropine-like activity in these drugs.

Atropine effectively antagonized both TMN- and OTMN-hypothermia and analgesia. Propranolol, PhQA33 and LB-46 antagonized TMN-tremor, hypothermia and analgesia, but afforded protection only against OTMN-tremor and not against OTMN-hypothermia and analgesia (Table 2). Hermansen (1968) also found that propranolol did not affect OTMN-hypothermia and analgesia in mice, while as in this study, others (Agarwal & Bose, 1967; Achari & Sinha, 1968; Cox & Potkonjak, 1970) have reported a blockade of OTMN-tremor. It is possible that propranolol exerts its anti-OTMN-tremor action at a peripheral site. However, the observed antagonism of TMN-tremor, hypothermia and analgesia by propranolol contradicts this view.

None of the compounds except atropine offered protection against physostigmine-induced death, which indicates an absence of a strong central anticholinergic activity. There is a debatable indication in the literature that a catecholamine-acetylcholine imbalance is involved in TMN and OTMN action (Spencer, 1965, 1966; Cox & Potkonjak, 1970). This adrenergic link may in some complex way be involved in the tremorlytic action of β-blockers, as Klinge & Aro (1971) have shown propranolol, alprenolol and oxprenolol to lower the adrenaline and raise the noradrenaline content (dopamine concentrations remain unchanged) of rat whole brain.

Acknowledgements

The generous gift of the following drugs is gratefully acknowledged: propranolol from ICI, U.K.; isomers of H56/28 from A.B. Hassle, Goteberg; LB-46 from Sandoz Ltd., Basle; PhQA33 from Pharmacia AS, Vanlose, Denmark; Kö-592 from Boehringer Ingelheim, W. Germany and isomers of INPEA from Selvi & Co., Milan.

REFERENCES

ÅBLAD, B., BROGARD, M. & EK, L. (1967). *Acta pharmac. tox.*, **25**, *Suppl.* 2, 9–40.
ACHARI, G. & SINHA, S. P. (1967). *Jap. J. Pharmac.*, **17**, 679–680.
ACHARI, G. & SINHA, S. P. (1968). *Ibid.*, **18**, 370–371.
AGARWAL, S. L. & BOSE, D. (1967). *Br. J. Pharmac. Chemother.*, **30**, 349–353.
BARTOLINI, A. & PEPEU, G. (1970). *Pharmac. Res. Comm.*, **2**, 23–29.
CAMPBELL, L. B., HANIN, I. & JENDEN, D. J. (1970). *Biochem. Pharmac.*, **19**, 2053–2059.
CHEN, G. (1958). *J. Pharmac. exp. Ther.*, **124**, 73–76.
CONSTAS, C. (1962). *J. Neurol. Neurosurg. Psychiat.*, **25**, 116–121.
COX, B. & POTKONJAK, D. (1969a). *Br. J. Pharmac.*, **35**, 295–303.
COX, B. & POTKONJAK, D. (1969b). *Ibid.*, **35**, 521–529.
COX, B. & POTKONJAK, D. (1970). *Ibid.*, **38**, 171–180.
EVERETT, G. M. (1956). *Nature, Lond.*, **177**, 1238.
FUNCKE, A. B. H., MULDER, D., VAN BEEK, M. C., TIMMERMAN, H., VAN HELL, G., LAVY, U. I. & NAUTA, W. T. (1969). *Archs int. Pharmacodyn. Thér.*, **177**, 28–51.
GIUDICELLI, J., SCHMITT, H. & BOISSIER, J. R. (1969). *J. Pharmac. exp. Ther.*, **168**, 116–126.
HAHN, R. A., PENDLETON, R. G. & WARDELL, JR., J. R. (1968). *Ibid.*, **161**, 111–121.
HERMANSEN, K. (1968). *Acta pharmac. tox.*, **26**, 343–362.
HERRING, A. B. (1964). *Lancet*, **2**, 892.
KHANNA, N. K. & MADAN, B. R. (1973a). *Ann. Indian Acad. Med. Sci.*, **9**, 113–119.
KHANNA, N. K. & MADAN, B. R. (1973b). *Indian J. med. Res.*, **61**, 1062–1065.
KLINGE, E. & ARO, S. (1971). *Eur. J. Pharmac.*, **14**, 124–129.
MARSDEN, C. D., FOLEY, T. H., OWEN, D. A. L. & MCALLISTER, R. G. (1967). *Clin. Sci.*, **33**, 53–65.
MARSDEN, C. D. & OWEN, D. A. L. (1966). *Am. J. Cardiol.*, **18**, 484.
MARSHALL, J. & SCHNEIDEN, H. (1966). *J. Neurol. Neurosurg. Psychiat.*, **29**, 214–218.
MILLER, L. C. & TAINTER, M. L. (1944). *Proc. Soc. exp. Biol. Med.*, **57**, 261–264.
NOSE, T. & KOJIMA, M. (1970). *Eur. J. Pharmac.*, **10**, 83–86.
OWEN, D. A. L. & MARSDEN, C. D. (1965). *Lancet*, **2**, 1259–1262.
SHARMA, J. N., SINGH, G. B. & DHAWAN, B. N. (1971). *Jap. J. Pharmac.*, **21**, 675–677.
SHARMA, P. L. (1970). *Q. Jl. exp. Physiol.*, **55**, 202–206.
SPENCER, P. S. J. (1965). *Br. J. Pharmac.*, **25**, 442–455.
SPENCER, P. S. J. (1966). *Life Sci.*, **5**, 1015–1023.
VAS, C. J. (1966). *Lancet*, **1**, 182–183.