EFFECT OF TEN BETA-ADRENOCEPTOR BLOCKING AGENTS ON SPONTANEOUS MOTILITY AND PENTOBARBITAL-INDUCED ANAESTHESIA IN MICE

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Summary: In sub-toxic doses, propranolol, both isomers of H 56/28, PhQA33 and LB-46 reduced spontaneous motor activity (SMA), while pronethalol and the isomers of INPEA enhanced it. Pentobarbitone-induced anaesthetic time was reduced by the isomers of INPEA but was increased by the remaining compounds. These actions permit categorization of beta-adrenoceptor antagonists into CNS stimulants and depressants. The insomers of INPEA appear to be CNS stimulants and the remaining compounds CNS depressant. Pronethalol was an exception and had opposite actions in the two test-procedures.

Key words: beta-adrenoceptor blockers spontaneous motor activity pentobarbitone-induced anaesthesia

INTRODUCTION

Propranolol was shown by Leszkovszky and Tardos (7) to possess a sedative property in rats and mice and evidenced by its ability to enhance hexobarbitone-induced anaesthetic time and reduce amphetamine toxicity. In confirmation of this, it was reported that patients receiving propranolol have a high incidence of depression (12), although this finding was questioned by Fitzgerald (4). There are many beta-adrenoceptor blocking agents and it is desirable that their actions on central nervous system be investigated. In this study the sedative activity of ten beta-adrenoceptor antagonists of diverse chemical structure administered in sub-toxic doses was tested.

MATERIALS AND METHODS

Test Drugs

The drugs used were: (±) *propranolol* HCl; (±) *pronethalol* HCl; dextro-1-(2-allylphenoxy -3-isopropylaminopropanol (-2) HCl (*d-H*56/28, d-alprenolol); laevo-1-(2-allylphenoxy)-3-isopropylaminopropanol (-2) bitartrate monohydrate (l-H56/28, l-alprenolol); (±) -1-(3-methylphenoxy)-2-hydroxy-3-isopropylaminopropane HCl (Ko-592, ICI-45,763); (±)-1-(isopropylamino) -3-(o-phenoxyphenoxy)-2-propanol HCl $\frac{1}{2}H_2O$ (*PHQA* 33); (±)-4-(2-hydroxy-3-isopropylaminopropoxy)-indole (*LB*-46); D, L (±) -1- (4-nitrophenyl) -2- isopropylaminoethanol HCl (D, L (±) INPEA); D(-) -1-(4-nitrophenyl) -2- isopropylaminoethanol HCl (D(-) INPEA); L(+)-1-(4-nitrophenyl)-2-isopropylaminoethanol HCl (L(+)INPEA) and pentobarbitone sodium (Abbott).

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In the text the drugs are referred to by the names underlined. All the compounds w 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, exc LB-46 which was used as a base.

Spontaneous motor activity (SMA): The method described by Dews (3) was followed essential details, employing the Asso Automatic Photocell Activity cage, Model 3A. Adu male, albino mice weighing 20-35 g were used. Experiments were performed in a dark and qui room maintained at a temperature of 20-24°C. To minimise the effect of diurnal variati in SMA, the tests were perfomaed at the same hour each day. Compounds were injected i dose of 60 *mg/kg* ip in groups of 5 mice and placed 10 min later on the runway. Coun per 5 mice were recorded every 10 min. for 90 min (nine counts). Mean cumulative activi per 90 min. was also recorded Control SMA records in 10 mice injected with a matchi volume of 0.9% saline ip were simliarly taken.

Pentobarbitone induced anaesthesia: Albino mice of either sex weighing 20-30 *g* wer used. An anaesthetic dose of pentobarbitone (60 *mg/kg* i.p.) was administered to groups o 10 mice. Test compounds in doses ranging between 5 to 70 *mg/kg* i.p. were administere in groups of 10 mice, 15 min prior to the administration of pentobarbitone in "test groups" Anaesthetic time in min was estimated as the time interval between the loss and the reappearanc of the "righting reflex" for each animal. Mean anaesthetic time for the control and the te group was computed. The relative potencies were expressed as the ED_{100} values i.e. th dose inducing a 100% increase in the sleeping time. The ED_{100} values were derived fro a semi-logarithmic plot of the doses against the observed anaesthetic time (6). The significanc at the dose-step 40 *mg/kg* i.p. was determined by applying the Student's test at the 5% lev ($P<0.05$).

TABLE I: Effect of some beta-blockers on the SMA of mice. The compunds were administered in a fixed dose of 60 *mg/kg* ip and movements recorded for 90 min.

*Compound**	*Mean cumulative activity per 90 min*	*SMA % of control*
—	1545.9 ± 56.6	—
Propranolol	876.4 ± 44.2	56.7
Pronethalol	1712.1 ± 57.5	110.7
d—H56/28	841.4 ± 52.4	54.4
l—H56/28	1185.2 ± 54.2	76.3
Ko—592	1206.5 ± 51.5	78.1
PhQA33	531.4 ± 48.2	34.4
LB—46	829.2 ± 47.5	53.7
DL(±)INPEA	1905.6 ± 55.5	123.2
D(—)INPEA	1780.4 ± 49.3	115.1
L(+)INPEA	1970.2 ± 54.8	127.4

*n = 10 for control and 5 for test values.

Acute intraperitoneal toxicity (LD 50): LD_{50} was determined in adult albino mice of either sex weighing from 20 to 30 *g*, by the method of Miller and Tainter (9). Test compounds dissolved in saline, in doses graded between 14 and 300 *mg/kg* in a geometric progression (geometric factor 1.4) were administered ip in groups of 5 mice at each dose level in a volume of 0.1 *ml*/10 *g* The animals were observed and deaths recorded upto 24 hr and 7 days.

RESULTS

It was observed that both in the control and test groups of mice, the SMA was high initially and declined later on. Propranol, d-H56/28, 1-H56/28, Ko-592, PhQA33 and LB-46 reduced the SMA. Maximum reduction of 65.6% was induced by PhQA33. In contrast, D,L(±) INPEA, D(-) INPEA, L(+) INPEA and pronethalol increased the SMA. Maximum increase of 27.4% was induced by L(+) (INPEA Table I).

TABLE II: Effect of some beta-adrenoceptor blocking compounds on pentobarbitone-induced asaesthesia in mice and their LD_{50} values.

Compound	*Dose* mg/kg ip*	*Mean anaesthetic time min ± SEM*		*% Prolongation*	*ED_{100} mg/kg ip*	*'P' value*	*LD_{50} ± SEM mg/kg ip*
		control	*test*				
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	10		22 ± 6.9	47			132 ± 13.3
Propranolol	20	15 ± 1.73	29 ± 7.7	93	23	<0.001	(106—158)
	40		42 ± 4.8	180			
	10		31 ± 6.3	34			
Pronethalol	20	23 ± 3.16	40 ± 3.5	73	30	<0.001	102 ± 8.6
	40		56 ± 2.8	143			(85—119)
	50		63 ± 7.5	174			
	5		26 ± 3.0	30			
d-H56/28	10	20 ± 1.14	31 ± 3.5	55	18	<0.001	76 ± 7.5
	20		41 ± 2.8	105			(61—91)
	40		61 ± 7.9	205			
	10		21 ± 4.5	5			
l-H56/28	20	20 ± 1.14	25 ± 3.6	25	47	<0.001	174 ± 16.1
	40		36 ± 5.2	80			(152—205)
	50		41 ± 5.5	105			

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Ko-592	10 20) 40) 50) 70	32 ± 4.68	34 ± 3.8 40 ± 3.8 59 ± 8.9 55 ± 7.8 66 ± 7.0	6 25 59 72 106	64	>0.05	151 ± 13.4 (128—181)
PhQA33	10 20 40 50	32 ± 2.82	51 ± 7.5 57 ± 6.8 69 ± 8.8 74 ± 9.5	59 78 115 131	33	<0.001	112 ± 9.5 (94—131)
LB-46	10 20 40 50	15 ± 1.73	21 ± 4.6 26 ± 3.5 36 ± 2.2 41 ± 5.1	40 73 140 173	30	<0.001	166 ± 14.1 (138—194)
DL(±)INPEA	40	23 ± 3.16	18 ± 1.4	—21	—	>0.05	186 ± 16.4 (154—218)
D(—)INPEA	40	28 ± 4.12	18 ± 2.8	—35	—	>0.05	178 ± 15.7 (147—209)
L(+)INPEA	40	23 ± 3.16	13 ± 1.1	—43	—	>0.01	174 ± 14.5 (145—202)

*n = 10 at each dose-step.
ED_{100} : Dose causing a 100% increase in anaesthetic time.
'P' value was obtained by using Student's t test at the dose-step 40 *mg/kg* i p.
Numerals in parentheses dentote the 95% confidence limits; Miller and Tainter (8).
Numberals preceded by minus signs denote % decrease in sleeping time.

Propranolol, pronethalol, d-H56/28, l-H56/28, PhQA33 and LB-46 induced a statistically significant prolongation of the anasethetic time. The increase caused by Ko-592 was insignificant ($P>0.05$). Comparison of the ED_{100} values indicated that d-H56/28 was the most potent compound in this respect. The findings are summarised in Table II. The INPEA compounds shortened the anaesthetic time; and of these L(+) INPEA induced a statistically significant decrease.

The LD_{50} values are shown in Table II. The INPEA compounds induced a dose-dependent motor inco-ordination excitement and hyperactivity. With the other compounds the animals were sedated and were listless till terminal convulsions. With all the compounds, death was preceded by convulsions, marked increa e in respiratory rate and exhaustion.

DISCUSSION

Grana and Sossi (5) studied the relationship between chemical structure and CNS effects of some beta-adrenoceptor antagonists. They inferred that compounds of the phenylethanol series like DCI, INPEA and sotalol (MJ-1999) were either stimulants or were devoid of any central activity, while the naphthylethanolamines and naphthoxypropanolamines depressed the central nervous system. The effect of beta-receptor antagonists included in this study as observed on SMA in mice does not support this viewpoint. Both the isomomers of H56/28 and PhQA33 depress SMA, i.e. they have a propranolol-like effect, although they possess chemical structures in which the bicyclic ring has been replaced by the allylphenoxy and phenoxyphenoxy groups respectively. Thus, it seems, that the CNS-depressant activity is not restricted to the naphthoxy moiety in propranolol. If increase or decrease in SMA is considered as an evidence for CNS stimulant and depressant avtivity respectively (2,3,5) our finding that pronethalol enhances SMA stands in contrast to that of Grana and Sossi (5) who have categorised it as a central depressant. Similar contradictory findings have been obtained with DCI. Mnnear and Rudzik (8) reported DCI to possess CNS-stimulant activity, while Hermansen (6) observed a depressant effect. The inability of INPEA to decrease SMA is in accordance with the findings of previous workers (6, 10). INPEA and its optical isomers exhibited a mild CNS-stimulant action. The dextro-isomer of H56/28 is devoid of beta-blocking activity (1). However, both the isomers of H56/28 decreased SMA. This corroborates the findings of Leszkovszky and Tardos (7), Murmann *et al.* (10) and Hermansen (6) who observed a lack of correlation between beta- blocking and CNS-depressant activitiy.

Almost a similar categorisation of the ten beta-receptor antagonists into stimulants and depressants of the CNS is evident from the results obtained in the pentobarbitone induced anaesthetic test and the acute intraperitoneal toxicity studies. The only exception is pronethalol which prologed hypnosis and thus exhibited opposite actions in the two test-procedures. It may be interpreted to mean that modification of barbiturate anaesthesia is not a firm evidence for stimulant or depressant actions of a compound on the CNS. It is possible that potentiation by pronethalol of pentobarbitone induced anaesthesia is independent of its CNS-stimulant action, and is attributable to factors like interference with enzymatic breakdown of the barbiturate or hypothermia (6). However, no direct evidence in support of this viewpoint is available or adduced in the present study.

In the assessment of the central nervous effects of drugs, the dose employed is important, as in high doses compounds exhibit central effects which are their toxic manifestations. According to Ther (11) an effect becomes significant if attained with a dose of 1/2 to 1/5th of the LD_{50} i.e. the ratio of LD_{50} : ED_{100} falling between 2 and 5. This ratio reckoned

for propranolol, pronethalol, d-H56/28, l-H56/28, Ko-592, PhQA33 and LB-46 indic that all of them had a significant CNS-depressant action. The INPEA compounds failed potentiate barbiturate-induced anaesthesia.

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