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Tissue cholinesterase inhibition by propranolol and related drugs

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The effect of (±)-propranolol and some related drugs have been investigated on the cholinesterase (ChE) enzyme activity of heart and brain tissues of the rat. Brain homogenates hydrolysed more methacholine than benzoylcholine and the reverse was true for the heart tissue. In-vitro, (±)-, (+)- and (−)-propranolol, as well as its quaternary analogue, UM-272, all significantly inhibited heart and brain ChE. Timolol and sotalol, however, were less potent. In-vivo, (±)-propranolol (30 μmol kg^{-1}) significantly inhibited brain ChE activity in rats when compared with saline controls. It is inferred that propranolol inhibits brain and heart ChE enzyme in a non-stereoselective manner and that this cholinomimetic action could be involved in the mediation of some of its therapeutic effects.

The β-adrenergic blocking agent propranolol has been one of the most widely used drugs in disorders of the cardiovascular system. It has also been found to be useful in conditions related to the eye such as wide angle glaucoma and the central nervous system like psychosis, tremor and alcohol withdrawal states. However, many of the drug's therapeutic and pharmacological actions cannot be explained by its ability to block β-adrenoceptors; other ancillary properties have been proposed to explain these effects. Because of the overlapping of cholinergic and adrenergic innervation in many systems, drugs acting on one system are known to modify the activity of the other; thus it is likely that propranolol could modulate cholinergic activity, and this is supported by recent evidence from our laboratory. Bilateral vagotomy has been reported to attenuate the antiarrhythmic action of propranolol in dogs (Alkondon et al 1984). Also, the anticholinergic agent, atropine, has been shown to antagonize the ocular hypotensive effect of propranolol in rabbits (Alkondon et al 1986), reduce the antiaggressive effect of this drug in rats (Ray et al 1984) and inhibit the increase in airway resistance of normal and asthmatic subjects (MacDonald et al 1967). All this evidence indicates involvement of a cholinergic mechanism in propranolol pharmacodynamics. Recent observations from our laboratory also suggested the ability of propranolol to inhibit the cholinesterase (ChE) enzyme of human plasma and red blood cells (Alkondon et al 1983). The present study is an attempt to evaluate the effect of propranolol and some related drugs on the ChE activity of heart and brain tissues with the aim of exploring the nature of cholinergic mediation in the cardiovascular and central nervous system effects of propranolol.

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Methods

Wistar rats (150–200 g) of either sex had free access to food and water until the morning of the day of the experiment when they were killed by cervical dislocation, the heart and the brain removed immediately, washed in ice cold phosphate buffer (pH 8·0) and separately homogenized in 7 ml of buffer. The homogenates were centrifuged at 10 000 rev min^{-1} at 0 °C for 10 min. The supernatant was tested for cholinesterase enzyme activity, which was determined photometrically (Pilz 1974). The protein content was also estimated (Lowry et al 1951). Methacholine

chloride and benzoylcholine iodide were used as substrates for determining true and pseudo ChE activity, respectively, and expressed as μmol of substrate hydrolysed (mg protein)$^{-1}$ min^{-1}.

The drugs and chemicals used were: (±)-, (+)- and (−)-propranolol hydrochloride (all from ICI, London), UM-272 iodide (pranolium; [*N,N*-dimethyl-1-isopropylamino-3-(1-naphthyloxy)-propan-2-ol) (G. D. Searle and Co. Illinois), timolol maleate (Merck, Sharp and Dohme, New Jersey) sotalol hydrochloride (Mead Johnson, Indiana) and methacholine chloride and benzoylcholine iodide (both from Sigma Chemicals, St Louis). All drugs were dissolved in distilled water for in-vitro experiments, whereas (±)-propranolol was dissolved in 0·9% NaCl (saline) and administered intraperitoneally in a volume of 5 ml kg^{-1}, for the in-vivo studies.

Results

In the saline-treated (control) group of rats, homogenates of brain hydrolysed methacholine more effectively than benzoylcholine, whereas the reverse was true for the heart. The methacholine hydrolysed (mean ± s.e.m.) was 17·7 ± 1·5 μmol (brain n = 15 animals) and 1·2 ± 0·5 nmol (heart n = 6 animals) (mg protein)$^{-1}$ min^{-1}. On the other hand, the benzoylcholine results were 0·7 ± 0·2 and 7·9 ± 0·7 nmol (mg protein)$^{-1}$ min^{-1}, respectively. These results indicate a predominance of true ChE activity in rat brain (25 times more than that of the pseudo enzyme) and pseudo ChE activity in the rat heart (6·6 times more than that of the true enzyme).

Racemic propranolol, its isomers, the quaternary analogue UM-272, timolol and sotalol inhibited the rat heart and brain ChE enzyme in-vitro (Table 1). Both timolol and sotalol were less potent when compared with the other drugs in inhibiting these enzymes.

In rats treated with (±)-propranolol (30 μmol kg^{-1}, i.p., 1 h before death), the mean concentration of methacholine hydrolysed by brain homogenates was 11·2 ± 1·0 nmol (mg protein)$^{-1}$ min^{-1} (n = 6). These values were significantly less ($P < 0·01$, Student's *t*-test) when compared with the values from saline-treated animals (17·7 ± 1·5 nmol (mg protein)$^{-1}$ min^{-1}, n = 15), indicating the inhibitory effect of racemic propranolol on rat brain ChE activity in-vivo.

Table 1. Effect of propranolol and related drugs on cholinesterase enzyme activity of heart and brain tissues of the rat in-vitro.

	IC50 (M)	
Drugs	Heart	Brain
(±)-Propranolol	$6·2 \times 10^{-4}$	$1·0 \times 10^{-2}$
(+)-Propranolol	$2·9 \times 10^{-4}$	$>1·0 \times 10^{-2}$
(−)-Propranolol	$1·2 \times 10^{-3}$	$9·0 \times 10^{-3}$
UM-272	$2·6 \pm 10^{-3}$	$7·0 \times 10^{-3}$
Timolol	$>3·0 \times 10^{-3}$	$>1·0 \times 10^{-2}$
Sotalol	$>3·0 \times 10^{-3}$	$>1·0 \times 10^{-2}$

Each value is the mean of three experiments. Benzoylcholine and methacholine were used as substrates for determining ChE activity of heart and brain homogenates, respectively. IC50 = concentration of the drug needed to produce 50% inhibition of the enzyme (determined by interpolation from the dose-percentage inhibition curve).

Discussion

The present results clearly indicate the ability of (±)-propranolol to inhibit rat heart and brain ChE in-vitro and brain ChE of rats in-vivo. The inhibition of tissue ChE in-vitro was also shared by the isomers and the quaternary analogue of propranolol. The fact, that both timolol and sotalol, relatively pure β-blockers, produced a weak inhibition of ChE indicates that there is no relation between β-receptor blocking and ChE inhibitory action. This is supported by the observation that the (+)-isomer of propranolol, a weak β-blocker (Barret & Cullum 1968), was a more potent inhibitor of cardiac ChE than the (−)-isomer. In addition, UM-272, a propranolol analogue with no β-blocking ability (Schuster et al 1973), also inhibited the ChE activity of the heart. On the contrary, however, there appears to be a good relation between the cardiac ChE inhibitory action and the membrane stabilizing action for these drugs since (±)-propranolol, which possesses this action (Shanks 1976), exhibited greater ChE inhibitory effect than timolol or sotolol, both of which lack it (Shanks 1976).

The greater specificity of the heart homogenates for benzoylcholine and those of brain for methacholine indicate the predominant occurrence of pseudo ChE in the heart and true ChE in the brain of rats. Both (±)- and (+)-propranolol were more potent in inhibiting the pseudo ChE (heart) than true ChE (brain), in-vitro. This is in agreement with earlier studies from our laboratory, in which we reported the inhibitory effect of some β-blockers on plasma and red blood cell ChE (Alkondon et al 1983).

The cholinomimetic action (ChE enzyme inhibition) of propranolol is interesting in view of the fact that its antiarrhythmic action (Alkondon et al 1984), ocular hypotensive action (Alkondon et al 1986) and antiaggressive effect (Ray et al 1984) are antagonized by either interrupting the cholinergic innervation (vagotomy) or by prior administration of atropine. The possibility of achieving such high concentrations of propranolol in the system is not unlikely since large doses (higher than those needed to produce β-receptor blockade) of this drug are needed to antagonize ventricular arrhythmias in animals (Barrett & Cullum 1968; Apantaku et al 1975) and in man (Woosley et al 1977). Moreover, the concentrations of β-blockers needed to produce an ocular hypotensive action in animals and in glaucoma patients are in the range of 0·1 to 1·0% solutions (Radius et al 1978; Zimmerman & Boger 1979) which can reach a local concentration of >1 mM on the surface of the eye, and this is compatible with the enzyme

inhibition theory. Further, higher concentrations of propranolol were also required to produce central nervous system effects such as anti-aggressive action in rats (Ray et al 1984) and antipsychotic actions in man (Yorkston et al 1977). In fact, the dose of propranolol (30 μmol kg^{-1}), which exhibited an anti-aggressive action (Ray et al 1984) did inhibit brain ChE enzyme activity after systemic administration, in the present study. In addition, propranolol has been shown to be accumulated in the myocardium and brain at concentrations several times higher than in plasma (Myers et al 1975: Schneck et al 1977). All this evidence strongly suggests a cholinergic mechanism (through ChE enzyme inhibition) in the mediation of some of the cardiovascular, central nervous system and ocular actions of propranolol.

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The adenosine receptor antagonist, 8-phenyltheophylline, causes diuresis and saliuresis in the rat

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The diuretic and adenosine antagonist actions of two alkylxanthines have been compared in the conscious rat. 8-Phenyltheophylline (10 mg kg^{-1}) antagonized adenosine-induced bradycardia in the rat for at least 3 h whereas enprofylline (10 mg kg^{-1}) had no effect on this response. 8-Phenyltheophylline (10 mg kg^{-1}) evoked diuresis and saliuresis in the rat whereas enprofylline (10 mg kg^{-1}) had no effect on excretory parameters. These results indicate that the diuretic action of some alkylxanthines may be related to adenosine antagonism.

The diuretic action of the alkylxanthines has been known for many years (Schmiedeberg 1905), however, the pharmacological basis for this effect has not been elucidated. One possibility is that the adenosine antagonist actions of some alkylxanthines are responsible for their renal effects, since adenosine is known to reduce urine volume and sodium excretion (Osswald 1975). In the present study we have examined this possibility by comparing the diuretic effect of an alkylxanthine with a high affinity for adenosine receptors (8-phenyltheophylline) with one which has low affinity for these receptors (enprofylline) (Collis et al 1984).

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Methods

Evaluation of adenosine antagonism in the conscious rat. Alderley Park Wistar rats were anaesthetized with Halothane and vinyl catheters were surgically implanted in the right jugular vein and thoracic aorta (via the left carotid artery). After a recovery period of 24 h, the aortic blood pressure was recorded directly via a pressure transducer (Bell and Howell L221) and displayed on a chart recorder (Devices MX2). Heart rate was derived from the blood pressure trace. Adenosine,