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MODIFICATIONS BY PROPRANOLOL OF THE RESPONSE OF ISOLATED RABBIT ATRIA TO ENDOGENOUS AND EXOGENOUS NORADRENALINE

BY

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Propranolol has a specific blocking action on beta receptors for adrenaline (Black, Crowther, Shanks, Smith & Dornhorst, 1964), has antiarrhythmic (Lucchesi, Whitsitt & Brown, 1966) and antifibrillatory properties (Benfey & Varma, 1966 ; Rowlands, Howitt & Markman, 1965) and is effective in relieving symptoms of angina pectoris (Keelan, 1965 ; Gillam & Prichard, 1965). The autonomic innervation of the heart may be involved in cardiac arrhythmias, fibrillation, increase in cardiac work, and coronary vasoconstriction, but little is known about the interactions between propranolol and the cardiac autonomic innervation.

Benfey & Varma (1964) showed that propranolol competitively inhibits an increase in force and rate of beat of isolated guinea-pig atria produced by noradrenaline but non-competitively inhibits the response to tyramine and butyrylcholine. Desmethylimipramine, which inhibits uptake of noradrenaline by storage sites (Iversen, 1965 ; Titus, Matussek, Spiegel & Brodie, 1966), potentiates the increase in heart rate induced by sympathetic nerve stimulation and prolongs the duration of the response (Toda, Matsuo and Konishi, unpublished). We have investigated the blocking action of propranolol on atrial responses to noradrenaline released by nerve stimulation and by tyramine in comparison with the responses to exogenous noradrenaline.

METHODS

Forty-eight albino rabbits of either sex, weighing 1.8–2.2 kg, were used. Under ether anaesthesia the sympathetic nerves, vagi and common carotid arteries were dissected free from surrounding tissues along the cervical trachea. The animals were killed by bleeding from the common carotid arteries, and a block of tissue including sympathetic nerves, vagi, heart, and adherent tissues was isolated postvertebrally as previously described (Toda & West, 1967 ; Toda & Shimamoto, 1968). The ventricles were discarded. Atria with attached sympathetic nerves were prepared in warmed, oxygenated nutrient solution. In some experiments conventional atrial preparations were used. The composition of the modified Ringer solution was as follows (m-moles/l.): Na, 162.1 ; K, 5.4 ; Ca, 2.2 ; Cl, 157.0 ; HCO_3, 14.9 ; dextrose, 5.6. The isolated atria were attached to a force-displacement transducer (Nihonkoden Kogyo Co.) and suspended in a 60 ml. muscle bath filled with the nutrient solution, gassed with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 30° ±0.5° C. All atria were allowed to equilibrate for 60 to 90 min before the addition of any drug.

One sympathetic nerve was lifted out of the solution, placed on a bipolar silver electrode and stimulated for 3 sec by a train of rectangular pulses of 1.0 msec duration at frequencies of 5/sec and 20/sec with supramaximal intensity. A monopolar silver electrode 0.5 mm in diameter and insulated to the tip was used for transmural electrical stimulation of intracardiac cholinergic and adrenergic nerve fibres innervating the S-A node and atria. This stimulation produces a decrease in force and rate of beat followed by an increase (Vincenzi & West, 1963; Lewartowski, 1963). Transmural stimulation with pulses of 0.1 msec duration and supramaximal intensity was applied at frequencies of 5, 20 and 100/sec for 2 sec using a Sanei type ES-103-Z pulse generator. The placing of the electrode in the optimal position for cardiac nerve stimulation has already been described (West & Toda, 1967).

Spontaneous rate and contractile force of atria were recorded on a Sanei two-channel pen-writer. Attention was not directed to contractile force measurements because it is dependent on atrial rate (Vane, 1957; Blinks & Koch-Weser, 1961). Atrial rate was estimated by taking the mean of ten measurements of intervals between contractions. After transmural stimulation of the cardiac nerve fibres, the maximal interval between contractions produced was measured. All results shown in figures and the text were mean values $\pm$ standard errors of the means. The significance of differences between means was calculated by Student's *t* test.

Drugs were added directly to the muscle bath to give cumulative increases in concentration. Only one series of concentration-response curves of tyramine was obtained from any one preparation. Atria were exposed to propranolol for 10 min before sympathetic and transmural stimulation, noradrenaline and tyramine were applied. Atria were exposed to desmethylimipramine in a concentration of 10^{-6} g/ml. for 30 to 60 min (eight experiments): the drug was then washed out.

The following drugs were used: *dl*-noradrenaline hydrochloride; tyramine hydrochloride; propranolol hydrochloride; and desmethylimipramine hydrochloride. The final concentrations in the muscle bath were expressed in g/ml. of the salts.

RESULTS

Sympathetic nerve stimulation

Increases in force and rate of beat after stimulation of sympathetic postganglionic fibres were dependent on frequency of stimulation in the range between 1 and 20/sec. Further increases in frequency did not give further increases in the effect.

The spontaneous rate of atrial preparations was slightly slowed by propranolol; mean values of the rate from nine preparations were 79.4 ± 3.9 beats/min without drug, 77.1 ± 4.5 beats/min with propranolol (10^{-7} g/ml.), 76.4 ± 3.1 beats/min with propranolol (3×10^{-7} g/ml.), and 72.6 ± 3.2 beats/min with propranolol (10^{-6} g/ml.). There was no significant difference between these values. An increase in rate of beat induced by sympathetic nerve stimulation was inhibited with increasing concentrations of propranolol. Fig. 1A illustrates the relationship between concentrations of propranolol, the increase in the atrial rate and the duration of the response produced by nerve stimulation at

Fig. 1. A: Effects of propranolol on the increase in atrial rate and the duration of the increase induced by sympathetic nerve stimulation. —, Rate increase; – – –, duration of the response; ○, response to nerve stimulation at a frequency of 20/sec ($N=9$); ●, response to nerve stimulation at 5/sec ($N=7$). B: Modifications by propranolol of the rate increase and the duration of the increase induced by sympathetic stimulation in atria treated with desmethylimipramine. ○, Response to nerve stimulation at 20/sec ($N=8$); ●, response to nerve stimulation at 5/sec ($N=7$). C: Effects of propranolol on the increase in atrial rate in response to exogenous noradrenaline. Noradrenaline in a concentration of: ×, 2×10^{-8} g/ml. ($N=8$); ○, 10^{-7} g/ml. ($N=8$); ●, 5×10^{-7} g/ml. ($N=8$); △, 2×10^{-6} g/ml. ($N=7$); ▲, 10^{-5} g/ml. ($N=6$). D: Effects of propranolol on the increase in atrial rate in response to tyramine. Tyramine in a concentration of: ×, 10^{-6} g/ml. ($N=7$); ○, 5×10^{-6} g/ml. ($N=7$); ●, 2×10^{-5} g/ml. ($N=7$); △, 10^{-4} g/ml. ($N=7$).

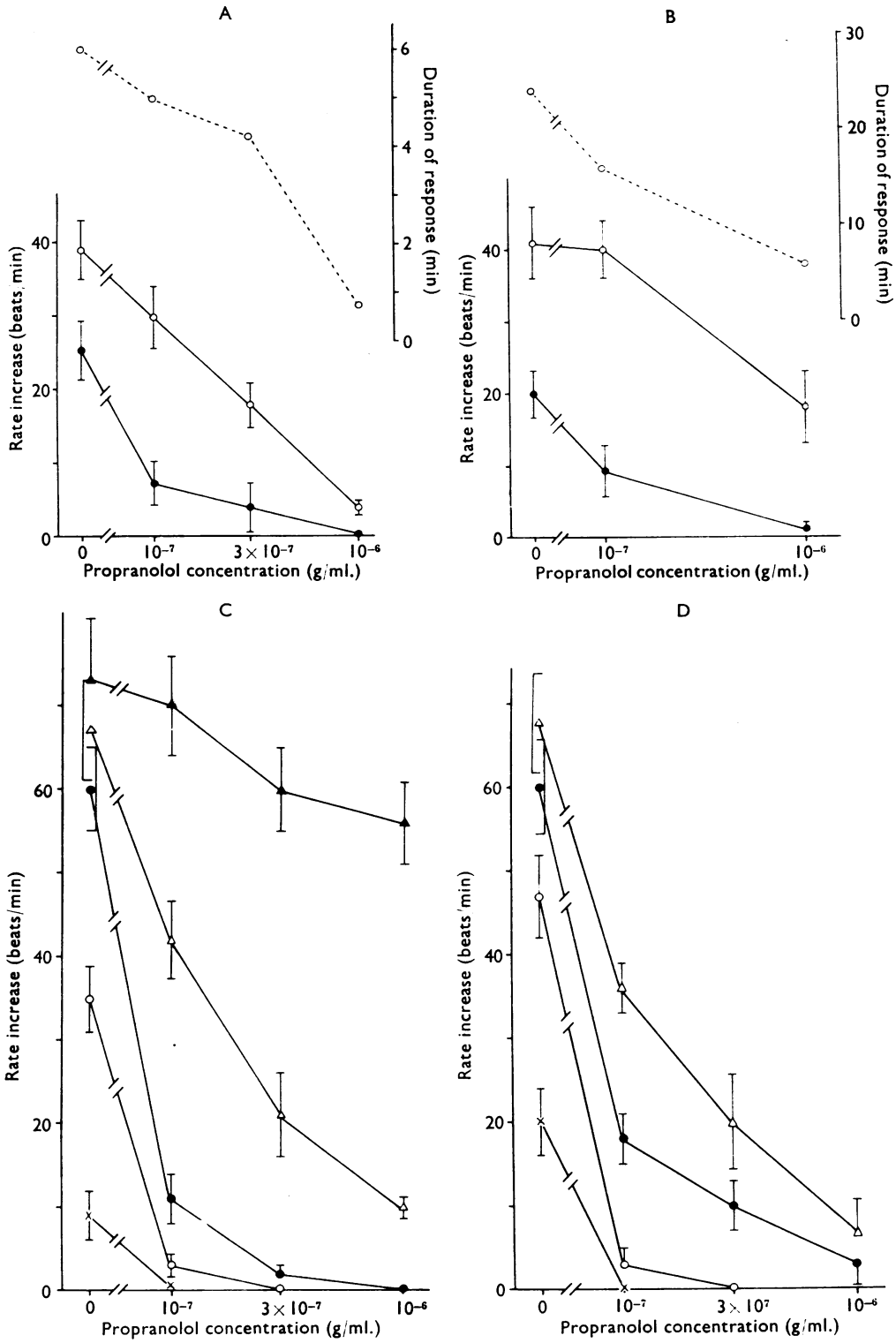

Fig. 1: See legend at foot of opposite page.

frequencies of 5 and 20/sec. The duration of the response was reduced in correspondence with the inhibition of the increase in rate. The increase in rate produced by the lower frequency of stimulation was more susceptible to propranolol than was the response to the higher frequency stimulation. The inhibitory effect of propranolol was not reversed by repeated washing with nutrient solution.

Desmethylimipramine

In preparations treated with desmethylimipramine (10^{-6} g/ml.) for 30 to 60 min and then bathed with fresh solution, the increase in rate induced by sympathetic stimulation was markedly prolonged, although the maximal increase in atrial rate was only slightly potentiated (Fig. 1B). The increase in rate produced by sympathetic nerve stimulation (20/sec) in those atria treated with desmethylimipramine was not affected by propranolol (10^{-7} g/ml.) but was moderately inhibited by 10^{-6} g/ml. The inhibitory action of propranolol on the effects of low frequency stimulation were not affected by treatment with desmethylimipramine (Fig. 1B).

Noradrenaline

An increase in rate produced by noradrenaline (2×10^{-8}–10^{-5} g/ml.) was inhibited in correspondence with increasing concentrations of propranolol. The results are shown in Fig. 1C.

Tyramine

Tyramine in cumulative concentrations of 10^{-6}, 5×10^{-6}, 2×10^{-5} and 10^{-4} g/ml. increased the atrial rate in proportion to the concentration. The rate increase was significantly diminished by propranolol (Fig. 1D). Susceptibility to propranolol of the effect of tyramine (5×10^{-6} and 10^{-4} g/ml.) was comparable with that of the effect of noradrenaline (10^{-7} and 2×10^{-6} g/ml.).

Comparison of effects of sympathetic nerve stimulation, noradrenaline and tyramine

A similar increase in atrial rate was induced in propranolol-free nutrient solution by sympathetic stimulation at a frequency of 20/sec, by noradrenaline (10^{-7} g/ml.) and by tyramine (5×10^{-6} g/ml.): that is, 39.1 ± 4.6, 35.0 ± 4.0 and 46.6 ± 6.2 beats/min, respec-

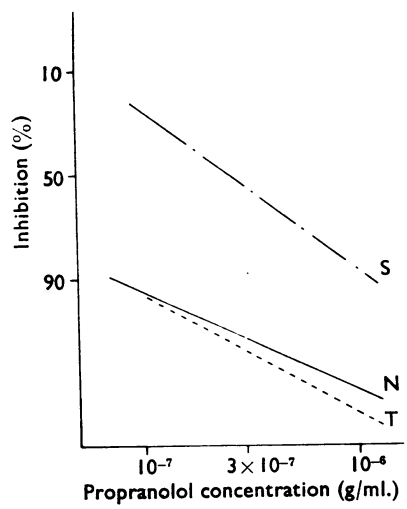

Fig. 2. Relationship between concentrations of propranolol and an inhibition of the effect of sympathetic nerve stimulation, tyramine and noradrenaline. S, Nerve stimulation at 20/sec; N, noradrenaline 10^{-7} g/ml.; T, tyramine 5×10^{-6} g/ml.

tively. Fig. 2 shows the inhibition by propranolol of these effects. Concentrations of propranolol inducing a 50% inhibition (ED50) of the effect were 2.5×10^{-7} g/ml. for sympathetic stimulation, 7×10^{-9} g/ml. for noradrenaline and 10^{-8} g/ml. for tyramine. With higher concentrations of noradrenaline (2×10^{-6} g/ml.) and tyramine (10^{-4} g/ml.) values of the ED50 of propranolol were 1.5×10^{-7} and 1.3×10^{-7} g/ml., respectively. Both of these values were less than the ED50 for nerve stimulation at 20/sec even though the amines produced a greater increase in atrial rate in the propranolol-free solution than did nerve stimulation (Figs. 1A, C and D).

Transmural electrical stimulation

Spontaneous rate of beat was decreased from 100 ± 7.5 beats/min ($N=6$) to 90.6 ± 8.4 beats/min ($N=6$, insignificant) by 10^{-6} g/ml. of propranolol and to 73.8 ± 9.0 beats/min ($N=6$, $P<0.05$) by 5×10^{-6} g/ml. of the drug.

A decrease in rate produced by transmural stimulation of intracardiac nerve fibres depended on the stimulation frequency. At frequencies of 20 and 100/sec the decrease in rate was inhibited by propranolol (5×10^{-6} g/ml.) (Fig. 3). At this concentration, however, propranolol may act as a local anaesthetic (Morales-Aguilerá & Vaughan Williams, 1965).

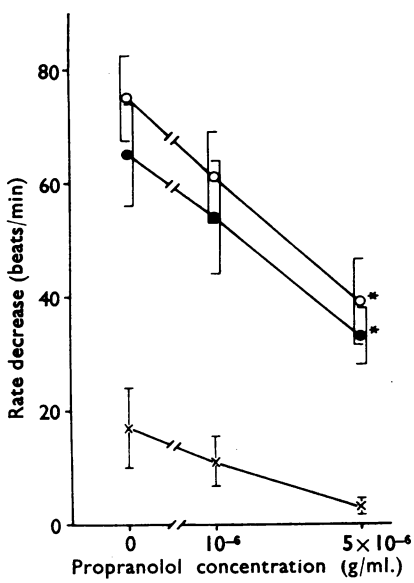

Fig. 3. Effects of propranolol on the decrease in rate produced by transmural stimulation of intracardiac nerves. Frequency: ○, 100/sec; ●, 20/sec; ×, 5/sec. Each point is the mean value from seven experiments. * Indicates significant difference from control, $P < 0.01$.

DISCUSSION

The increase in rate produced by stimulation of postganglionic sympathetic nerves at frequencies of 5 and 20/sec was inhibited with increasing concentrations of propranolol. Prior treatment of the atria with desmethylimipramine reduced the inhibitory action of propranolol. Desmethylimipramine inhibits the uptake of noradrenaline by sympathetic nerve terminals (Iversen, 1965; Titus *et al.*, 1966) and prolongs the duration of the increase in rate following sympathetic stimulation (Toda, Matsuo & Konishi, unpub-

lished). The prolongation of the duration may be related to a reduced rate of removal of noradrenaline from adrenergic receptors by uptake into storage sites, thus permitting high concentrations of cardiac noradrenaline to compete with adrenergic receptors with propranolol.

The increase in rate induced by sympathetic nerve stimulation was less susceptible to propranolol than was the response to tyramine and exogenous noradrenaline. Thus, the ED50 of propranolol for sympathetic stimulation at a frequency of 20/sec was about thirty times higher than for noradrenaline and tyramine in concentrations which produced a comparable increase in atrial rate. Yet with all these stimuli, the reaction is induced by activation of receptors by noradrenaline, applied exogenously or released from storage sites in the nerve terminals. Weiner, Draskóczy & Burack (1962) suggested that three catecholamine-containing compartments exist: (1) a small compartment, containing easily mobilized noradrenaline which is released by low frequency stimulation and possibly by tyramine ; (2) a storage compartment, the content of which can be released by intense nerve stimulation but not by tyramine ; and (3) a compartment containing bound noradrenaline which can be depleted by tyramine but not by nerve stimulation. Furthermore, pretreatment of guinea-pigs with reserpine 1.5 mg/kg completely abolishes an increase in rate of isolated atria to tyramine but does not influence that to sympathetic stimulation (Barnett & Benforado, 1966). Our results indicate that different mechanisms are involved in releasing cardiac noradrenaline by nerve stimulation and by tyramine. Differences of susceptibility to propranolol of the response to cardiac noradrenaline released by electrical and chemical stimulation might derive from an interference with access of the chemical stimulant to storage sites of noradrenaline and/or from a prevention of noradrenaline release from the tyramine compartment.

Propranolol inhibited the increase in rate produced by exogenous noradrenaline more effectively than the response to endogenous noradrenaline released by sympathetic stimulation. This effect is similar to the difference in the ability of atropine to block effects of exogenous acetylcholine and that released by the postganglionic parasympathetic nerves. One explanation of this is that it is "due to release of chemical mediator by the nerve near receptors in relatively inaccessible sites where diffusion limits the concentration of atropine" (Goodman & Gilman, 1965). This may also apply for propranolol.

Arrhythmias are induced experimentally by combined application of vagal stimulation and adrenaline. Acetylcholine shortens the refractory period of cardiac muscle and tends to induce arrhythmias and cardiac fibrillation. In the present study a decrease in rate produced by transmural stimulation of intracardiac nerves was only inhibited by high concentrations of propranolol. No inhibition of the decrease in rate produced by vagal stimulation was found after an infusion of propranolol in anaesthetized cats (Black, Duncan & Shanks, 1965). The antiarrhythmic and antifibrillatory activity of propranolol is probably not correlated with a diminution of parasympathetic tone but with an inhibition of sympathetic activity and a decrease in cardiac excitability (Morales-Aguilerá & Vaughan Williams, 1965).

SUMMARY

1. Atrial preparations with attached sympathetic postganglionic nerves and conventional atrial preparations from rabbits were used.

2. An increase in rate produced by sympathetic nerve stimulation at frequencies of 5 and 20/sec was inhibited by propranolol (10^{-7} to 10^{-6} g/ml.). The inhibitory action of propranolol was partly antagonized by treatment of atria with desmethylimipramine.

3. Concentrations of propranolol inducing a 50% inhibition (ED50) of an increase in rate induced by noradrenaline and tyramine were about one-thirtieth of the ED50 for sympathetic nerve stimulation.

4. Propranolol only inhibited the decrease in rate induced by transmural electrical stimulation of intracardiac nerve fibres at high concentrations.

5. It is concluded that propranolol antagonizes the beta receptor stimulating action of exogenous noradrenaline and of tyramine more effectively than the stimulating action of endogenous noradrenaline released by sympathetic nerve stimulation in isolated atria.

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