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THE USE OF SYMPATHETIC β-RECEPTOR BLOCKING AGENTS IN THE INVESTIGATION OF REFLEX CHANGES IN HEART RATE

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Pronethalol has been shown to have a highly specific blocking action on sympathetic β-receptors (Black & Stephenson, 1962). It should therefore provide an ideal physiological tool to block sympathetic nerves to the heart whilst leaving other pathways intact. However, the experiments described in this paper showed that pronethalol completely prevented the change in heart rate associated with the occlusion of the carotid arteries and may therefore have effects on reflex changes occurring through parasympathetic as well as sympathetic pathways.

Recently a new β-receptor blocking agent, propranolol, has become available. Effective β-receptor blockade was achieved with this drug at about one-tenth of the dose needed for pronethalol and the therapeutic ratio was about ten-times greater than that for pronethalol (Black, Crowther, Shanks, Smith & Dornhorst, 1964).

The experiments described in this paper show that in anaesthetized dogs propranolol effectively blocks the sympathetic nerves to the heart and may be used to differentiate between activity in efferent sympathetic and parasympathetic nerves to the heart.

METHODS

Dogs of 12 to 25 kg were given a subcutaneous injection of morphine sulphate (0.5 mg/kg). One hour later under local anaesthesia a catheter was inserted through a saphenous vein into the inferior vena cava and each animal was anaesthetized by an infusion of either a solution of chloralose (B.D.H.; dose 10 ml./kg of a 10-mg/ml. solution) in 0.9% saline, a steady state of light anaesthesia being maintained by the infusion of chloralose (about 10 mg/kg) every 15 min; or a solution of pentobarbitone sodium (Nembutal, Abbott Laboratories; dose 20 mg/kg), a steady state of light anaesthesia being maintained by the infusion of pentobarbitone (about 2 mg/kg) every 45 min. A tracheal cannula was inserted and the lungs were ventilated with air supplied from a Starling Ideal pump at a rate of 18 strokes/min; the stroke of the pump was adjusted to maintain the arterial carbon dioxide tension at 40±5 mm Hg. When the chest was opened a resistance to expiration was provided by placing the expiratory outlet from the respiratory pump under 3 cm of water. Loose snares were placed around the carotid arteries. The chest was opened by a mid-sternal incision and the right stellate ganglion and the right ansa subclavia were exposed. In some dogs all branches of the stellate ganglion were divided except for the ansa subclavia, in others a clamp was placed on the ansa subclavia to crush the nerve close to the stellate ganglion and left in position for 10 min. The right ansa subclavia was stimulated using bipolar silver electrodes shielded from all tissue except the nerve. Rectangular pulses were generated by a type S-4 Stimulator (Grass Instrument Co., U.S.A.) isolated from earth by an R.F. Isolation Unit (Grass Instrument Co., U.S.A.).

A short metal cannula (Inconel; Johnson, Matthey & Co., London; 1.5 mm bore), treated with a solution of dialkyl dimethylammonium chlorides (Arquad; Armour Hess) as a non-wetting agent, was placed in the right femoral artery. To the cannula was attached a Statham strain gauge (Model P23 Gb) the output of

which was connected to a carrier amplifier (S.E. Laboratories; Feltham, Middlesex) and the pressure was recorded on a direct-writing ultraviolet-light recorder (S.E. Laboratories; Feltham, Middlesex). The frequency response of the system obtained by the method of Linden (1959) was flat (±5%) to better than 60 cycles/sec. The manometer was calibrated in a stepwise manner by means of a mercury manometer; zero pressure was recorded *post mortem* as pressure at the cannula tip, with the tip in air, free of blood and other tissue. " Mean pressure " was obtained electrically by passing the output of the carrier amplifier through a simple RC network with a time constant of 1 sec. The electrocardiogram was recorded from the left foreleg and the right chest wall. Heart rate was recorded by means of a cardiotachometer (McCook & Peiss, 1959) triggered by the arterial pressure pulse; the output filter had a time constant of 8 sec. The rectal temperature was maintained at 37.5±1° C by adjusting heating lamps above and beneath the animal.

The carbon dioxide tension (P_{CO_2}) of arterial blood was measured in blood withdrawn anaerobically from a catheter in the left femoral artery with the tip in the abdominal aorta. Samples of 5 to 7 ml. of blood were withdrawn into the syringes in which the dead space was filled with a solution of heparin (Pularin; Evans Medical, 1,000 U/ml. in saline, 0.9% w/v). The blood was transferred immediately to a Severinghaus P_{CO_2} electrode (National Welding Co., Richmond, California, U.S.A.) the temperature of which was maintained at 38±0.05° C. The output of the electrode was measured using a 33B Vibron electrometer and C33B *p*H unit (Electronic Instruments, Richmond, Surrey, England); the system measured P_{CO_2} with an accuracy ($P=0.05$) of ±2.5% of the P_{CO_2}.

RESULTS

Effect of pronethalol on the response to occlusion of the carotid arteries. Five dogs were used in this experiment; four were anaesthetized with pentobarbitone and one was anaesthetized with chloralose. Both carotid arteries were occluded for a period of 30 to 40 sec on two occasions in four dogs and one occasion in one dog. An intravenous injection of pronethalol (1 to 5 mg/kg) was given and 5 to 15 min later the carotid arteries were again occluded. The average heart rates and arterial mean pressures before and after occlusion of the carotid arteries were compared with the heart rates and mean arterial pressures during the period between 10 to 20 sec after starting the occlusion. During each of the nine occlusions performed before the injection of pronethalol the heart rate increased (mean increase, 27 beats/min; range 5 to 44 beats/min) and the mean arterial pressure rose (mean increase, 52 mm Hg; range 30 to 80 mm Hg). After the injection of pronethalol (1 to 5 mg/kg), carotid occlusion in fourteen experiments resulted in no change in heart rate and in one experiment the increase in heart rate was 5 beats/min. In all experiments the blood pressure rose (mean increase, 32 mm Hg; range 20 to 55 mm Hg). During the control period, before the injection of pronethalol, if the occlusion of the carotid arteries was ended suddenly there was always an immediate and dramatic decrease in heart rate, the so-called " vagal slowing." This effect presumably resulted from the sudden increase in pressure in the carotid sinuses evoking a reflex response through the efferent vagus nerves. After the injection of pronethalol (1 to 5 mg/kg), " vagal slowing " was not seen in thirteen of the fifteen experiments; in the dog anaesthetized with chloralose, after the injection of pronethalol (1 mg/kg) " vagal slowing " was seen after two periods of carotid artery occlusion. However, this effect was not seen after a second dose of pronethalol (1 mg/kg) had been given.

The mean arterial pressure during occlusion of the carotid arteries was always less after the injection of pronethalol than during the control occlusions and this may explain the absence of " vagal slowing " after the carotid occlusion was released. Two dogs which had received 2 mg/kg of pronethalol were therefore given an intravenous infusion of adrenaline hydrochloride until the mean arterial pressure rose to 200 mm Hg. In one dog this increase in pressure was not accompanied by slowing of the heart and there was no slowing after

release of carotid artery occlusion performed whilst the mean arterial pressure was high. In the other dog the increase in pressure was accompanied by slowing of the heart rate and release of carotid artery occlusion performed whilst the mean arterial pressure was high resulted in a slowing of the heart.

Such evidence suggested that in some dogs, at least, pronethalol interfered with reflex changes in heart rate which were mediated not only through sympathetic pathways but also through efferent vagal pathways. That the vagal efferent pathway to the heart was not wholly blocked was shown by stimulating the cut peripheral end of the right vagus nerve after pronethalol; stimulation always caused immediate slowing of the heart rate. In two dogs pronethalol was also injected during the recording of afferent impulses in slips of the cervical vagus nerve; there was no obvious change in the impulse activity in fibres from lung stretch receptors or baroreceptors in the aortic arch.

Effects of an intravenous injection of propranolol. Nine dogs were used in this series of experiments, four anaesthetized with pentobarbitone and five with chloralose. The dogs were each given successive injections of propranolol in doses of 0.1, 0.5 and 3 mg/kg. The propranolol was dissolved in saline (0.9%, w/v) in a strength of 1 mg/ml. and injection was made rapidly into a catheter inserted through a femoral vein into the inferior vena cava. There were no immediate changes in heart rate or arterial pressure but in seven out of the nine dogs within about 30 sec the heart rate began gradually to decrease to reach a new steady level within about 5 min. The average heart rates in the nine dogs are shown in Table 1; these measurements were made 5 to 10 min after the injection of propranolol.

TABLE 1

EFFECT OF PROPRANOLOL ON HEART RATE

Average heart rates (beats/min) in nine dogs before injection of propranolol and 5 to 10 min after administration of the doses indicated

	Heart rate (beats/min)			
		After dose of propranolol (mg/kg)		
	Control	0·1	0·5	3
Mean	118	106	103	104
Standard error	±11·2	±8·0	±6·2	±5·8
Range	71–183	68–153	69–132	84–123

The experiments were carried out within 5 to 20 min after the injection of propranolol. rate; in dogs in which the initial heart rate was less than 120 beats/min there were comparatively small changes in heart rate. In addition, in two dogs in which the control heart rates were 97 and 87 beats/min the heart rate increased after propranolol (0.1 mg/kg) by 17 and 2 beats/min respectively. In six dogs there was no change in the mean arterial pressure even after a dose of 3 mg/kg of propranolol; a fall of mean arterial pressure of 10 to 15 mm Hg after a dose of 3 mg/kg of propranolol occurred in three dogs (for example, Fig. 1).

Effects of stimulation of the right ansa subclavia. In six dogs, three anaesthetized with pentobarbitone and three with chloralose, the right ansa subclavia was stimulated before and after the injection of propranolol. Two rates of stimulation were used, 15 shocks/sec which gave maximal effects (Ledsome & Linden, 1964a) and 3 to 5 shocks/sec which gave submaximal effects. The heart rate was counted over 30-sec periods before stimulation,

after the stimulation had been applied for 1 min, and 3 min after stopping the stimulation. The experiments were carried out within 5 to 20 min after the injection of propranolol. Practical difficulties, for example a need to give more anaesthetic in the middle of a run, meant that results of complete runs at each rate of stimulation were available in only four dogs. Table 2 shows the increases in heart rate during the periods of stimulation calculated by subtracting the average of the heart rates before and after stimulation from the heart rate during stimulation. The results for the control experiments are the average of two stimulations; those after the administration of propranolol are the results of one stimulation in each case. The administration of propranolol reduced the increase in heart rate produced by stimulation of the right ansa subclavia. It should be noted that the increase in heart rate produced by submaximal stimulation was almost completely abolished by 0.5 mg/kg of propranolol, whereas this dose reduced the effect of maximal stimulation by 75%. In one dog (Table 2, No. 17) there was still an appreciable increase in heart rate on stimulation of the ansa subclavia after a dose of 3 mg/kg of propranolol. To demonstrate that this was not due to stimulation of afferent nerves producing changes in the heart rate reflexly through vagal efferent pathways both vagus nerves were cut in the neck and the stimulation repeated; the heart rate then increased by 40 beats/min.

TABLE 2

EFFECT OF STIMULATION OF THE RIGHT ANSA SUBCLAVIA ON HEART RATE

Increases in heart rate (beats/min) in six dogs during stimulation of the right ansa subclavia before and after injection of propranolol in the doses indicated. Three dogs were anaesthetized with chloralose (Chlor.) and three with pentobarbitone (Pento). All results were obtained within 5 to 20 min of administration of propranolol. Submaximal stimulation was at 3 to 5 shocks/sec, 3 to 10 V and 2-msec duration. Maximal stimulation was at 15 shocks/sec, 5 to 10 V and 2-msec duration

		Heart rate (beats/min)			
			After dose of propranolol (mg/kg)		
Dog No.	Anaesthetic	Control	0·1	0·5	3
Submaximal stimulation					
27	Pento.	68	17	1	0
29	Chlor.	95	30	9	1
31	Pento.	70	33	6	0
33	Chlor.	57	4	0	0
Average		72	21	4	0
Maximal stimulation					
16	Pento.	72	43	16	2
17	Chlor.	126	89	37	30
29	Chlor.	140	100	33	6
33	Chlor.	131	76	28	3
Average		117	77	29	10

Recovery from propranolol. In two dogs (Table 2, Nos. 27 and 31) the effect of submaximal stimulation of the right ansa subclavia was studied in more detail. Two stimulations were made before the injection of propranolol, between 5 to 20 min after the injection of propranolol and between 45 to 60 min after the injection of propranolol. The average increases in heart rate in these experiments are shown in Table 3. The results indicate that there was some recovery from the effects of the injection of propranolol during the first hour after the injection.

TABLE 3

RECOVERY FROM PROPRANOLOL

Increases in heart rate (beats/min) produced by submaximal stimulation of the right ansa subclavia (5 V, 5 shocks/sec, 2 msec duration) before and at times shown after administration of propranolol. Figures are averages of two stimulations

	Heart rate increase (beats/min)				
		After dose of propranolol (mg/kg)			
		0·1		0·5	
Dog No.	Control	5–20 min	45–60 min	5–20 min	45–60 min
27	68	17	33	1	18
31	70	33	54	6	14

Effects of occlusion of both carotid arteries. In six dogs, three anaesthetized with pentobarbitone and three with chloralose, the carotid arteries were occluded before the injection of propranolol and 5 to 20 min after the injection of 0.1, 0.5 and 3 mg/kg of propranolol. The arterial pressure records in one of the dogs are shown in Fig. 1. The

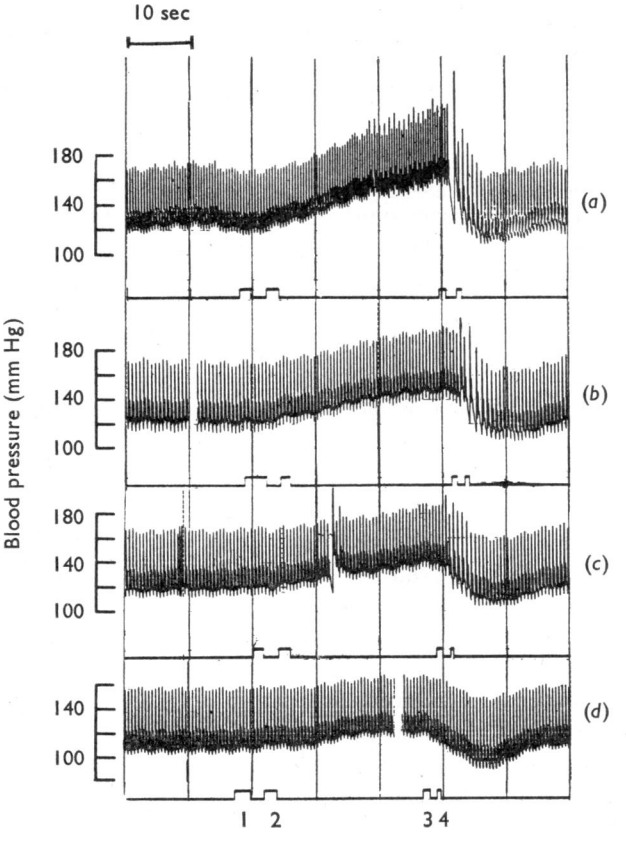

Fig. 1. Parts of the femoral arterial pressure record from one experiment. In each record the carotid arteries were occluded at the signal marks 1 and 2 and at the signal marks 3 and 4 the occlusion was released. Records from above downwards, control before injection of propranolol (*a*), 5 min after injection of propranolol, 0.1 mg/kg (*b*), 5 min after injection of propranolol, 0.5 mg/kg (*c*), and 5 min after injection of propranolol, 3 mg/kg (*d*).

results of the occlusions made at each stage are shown in Table 4. After the injection of propranolol (0.1 and 0.5 mg/kg) all six dogs showed an increase in heart rate when both carotid arteries were occluded and an immediate " vagal slowing " for two or three beats when the occlusion was released, thus demonstrating a vagal component of the reflex response. After a dose of 3 mg/kg of propranolol the changes in mean arterial pressure were less; in four dogs (three anaesthetized with pentobarbitone and one with chloralose) there was no change in heart rate either during occlusion or on release of occlusion; in the other two dogs there was an increase in heart rate during carotid occlusion and a slowing when the occlusion was released.

TABLE 4

EFFECTS OF OCCLUSION OF THE CAROTID ARTERIES

Increases in heart rate and in mean arterial pressure during fifty occlusions of the carotid arteries in six dogs before and after the injection of propranolol in the doses indicated. Values are means $\pm$ standard errors

Dose of propranolol (mg/kg)	Heart rate increase (beats/min)	Mean blood pressure increase (mm Hg)
Control	20$\pm$4·3	46$\pm$4·4
0·1	12$\pm$3·5	36$\pm$6·8
0·5	11$\pm$3·4	29$\pm$6·3
3·0	6$\pm$3·6	20$\pm$7·7

DISCUSSION

In experimental animals changes in heart rate may occur reflexly as the result of changes in activity in the sympathetic or parasympathetic nerves to the heart. To distinguish between activity in efferent sympathetic or parasympathetic nerves it would be an advantage if an agent were available which would prevent the action of the sympathetic nerves without interfering with reflex changes mediated through parasympathetic pathways. Bretylium tosylate may be used for this purpose (Ledsome & Linden, 1964a) but this drug causes a marked sympathomimetic effect and it may be 15 min to 2 hr before the heart rate and arterial pressure return to their original levels; only after this time is it valid to compare the effects of an experimental manoeuvre with the effects of a similar manoeuvre interposed during the control period.

Pronethalol has been shown to block the positive chronotropic and inotropic actions on the heart of injected catechol amines and sympathetic nerve stimulation (Black & Stephenson, 1962). It has been used to examine the effects of sympathetic blockade of the heart on the circulatory response to exercise (Dornhorst & Robinson, 1962; Chamberlain & Howard, 1964). The dose of pronethalol which effectively blocks the sympathetic nerves to the heart is uncertain but Black & Stephenson (1962) found that 2.5 mg/kg caused a 57% reduction in isoprenaline-induced tachycardia and 5 mg/kg caused a 90% reduction in the tachycardia caused by stimulation of the stellate ganglion. In the experiments described here an injection of 1 mg/kg usually, and 2 mg/kg always, prevented the increase in heart rate resulting from the occlusion of the carotid arteries. Even if these small doses completely blocked the sympathetic nerves to the heart in these animals, it should still have been possible to demonstrate reflex changes in heart rate mediated through parasympathetic efferent pathways. The failure to demonstrate such changes may possibly be explained by the fact that there was a smaller increase in arterial pressure during carotid artery occlusion after the infusion of pronethalol. Thus the sudden stimulus to the carotid

sinus baroreceptors brought about by releasing the occlusion would be less. However, it does seem that this explanation may not be the only one: in one animal out of two, raising the blood pressure after the infusion of pronethalol by the injection of adrenaline to a level above that observed in the control period and then releasing the occlusion of the carotid arteries did not slow the heart rate. This evidence suggests that a reasonable hypothesis is that pronethalol may prevent the action of some reflexes whose effect on the heart rate is mediated by the efferent vagal nerves. Therefore, although pronethalol is known to block effects attributable to sympathetic β-receptors, it is not a suitable agent with which to differentiate between activity in the efferent sympathetic and parasympathetic nerves to the heart. Also, changes in the cardiovascular response to exercise after administration of pronethalol should be interpreted with the knowledge that the injection of pronethalol may have effects other than a simple blockade of β-receptors. The explanation and site of the action of pronethalol on the vagal heart rate reflex are not known and the problem was not investigated further because a more potent agent (propranolol) became available.

Propranolol caused no sympathomimetic effect and after injection the heart rate decreased. The reduction in the range of heart rates after the injection of propranolol shows that in these anaesthetized animals some of the variations in heart rates resulted from differences in activity in the sympathetic nerves to the heart. Animals with slow heart rates of 70 to 90 beats/min had little or no change in heart rate on injection of propranolol and presumably at these heart rates in these animals there was no activity in the sympathetic nerves to the heart.

The increase in heart rate caused by stimulation of the right ansa subclavia was considerably reduced by propranolol. Before the injection of propranolol the increase in heart rate produced by submaximal stimulation of the ansa subclavia was 60 to 90 beats/min. Most reflex increases in heart rate observed in anaesthetized animals prepared similarly to those in these experiments are well within this range (for example, Ledsome & Linden, 1964b). Therefore it may be assumed that any reflexly produced changes in sympathetic activity to the heart are likely to be comparable with the effects of submaximal stimulation of the ansa subclavia rather than with maximal stimulation. Thus from the results described here it may be expected that an injection of 0.5 mg/kg of propranolol should prevent more than 90% of the reflex heart rate change mediated through the sympathetic nerves during the first 20 min after injection; after 1 hr there should still be a large reduction in sympathetic effects. Propranolol was less effective (about 75% reduction in response) in preventing the changes in heart rate caused by maximal stimulation of the right ansa subclavia than it was in preventing the effects of submaximal stimulation. This may indicate that propranolol competes with catechol amines for the receptor and that in the presence of a large concentration of catechol amine some effects of the catechol amines on the receptor will always be seen.

The increase in heart rate produced by occlusion of the carotid arteries is caused by changes in activity in both sympathetic and parasympathetic nerves (Ledsome & Linden, 1964a). Injection of propranolol in doses of 0.1 and 0.5 mg/kg reduced the increases in heart rate produced by carotid occlusion presumably because some or most of the increased sympathetic activity during occlusion was blocked. But even with doses of 0.5 mg/kg there was still a definite slowing of the heart rate when the occlusion was suddenly released, indicating that the carotid sinus vagal reflex was intact. However, after a dose of 3.0 mg/kg

of propranolol had been given in some of the experiments there was no change in heart rate on carotid occlusion or release of carotid occlusion. This effect was similar to that seen with pronethalol given in this dose; it is probable that the disappearance of reflex changes occurring through the parasympathetic nerves may be the result of an action unconnected with the action of these drugs upon β-receptors.

The experiments described here show that propranolol may be used in the anaesthetized dog to distinguish between changes in heart rate caused by changes in sympathetic nervous activity and those caused by parasympathetic nervous activity. Two doses may be used for different purposes. A dose of 0.5 mg/kg should prevent most (certainly more than 75% and probably more than 90%) of the change in heart rate caused by reflex changes in sympathetic nervous activity. A dose of 0.1 mg/kg may be used if it is desired to show only that sympathetic nerves to the heart were active, or if subsequent recovery from the drug were required.

SUMMARY

1. Injection of pronethalol in doses of 1 to 5 mg/kg prevented the change in heart rate caused by occlusion and release of occlusion of the carotid arteries.

2. The increase in heart rate caused by submaximal stimulation of the right ansa subclavia was reduced by the injection of 0.1 mg/kg of propranolol and almost abolished by the injection of 0.5 mg/kg of propranolol.

3. Injection of propranolol in doses of 0.1 to 0.5 mg/kg reduced but did not prevent the increase in heart rate caused by occlusion of the carotid arteries; the " vagal slowing " following sudden release of the occlusion was still present.

4. Propranolol in doses of 0.1 to 0.5 mg/kg may be used to distinguish between activity in sympathetic and parasympathetic nerves to the heart.

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