Beta-Adrenergic Blocking Effects of Propranolol.* (31566)

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The first specific beta-adrenergic blocking agent to be developed, dichloroisoproterenol (1,2), has complicating sympathomimetic properties(3). Black and Stephenson(4) introduced pronethalol which had potent beta-adrenergic blocking properties and, at least in isolated organs, had little or no sympathomimetic action. However, it was found to produce malignant tumors in mice. Further, it was not free of sympathomimetic effects, at least in the dog(5). Therefore it has been replaced with propranolol (Inderal†)(6). This investigation was designed to assess the beta-adrenergic blocking effects of propranolol in the intact, anesthetized dog.

Materials and methods. Dogs weighing 12.5 to 23 kg were anesthetized with a combination of morphine sulfate (2 to 3 mg/kg I.M.) and sodium pentobarbital (15 mg/kg I.V.) A catheter was introduced *via* an external jugular vein and placed with its tip in the right atrium. Pressure was measured with a Statham P23D strain-gauge transducer. Arterial pressure was recorded *via* a needle in the femoral artery. The zero reference point for pressures was half way between the dog's front and back when supine. Cardiac output was measured by the indicator dilution method. Indocyanine green dye was injected into the right atrium and blood was sampled at 38 ml/min by a Harvard pump through a Gilford cuvette densitometer. The sensitivity of the densitometer was adjusted so that a concentration of 10 mg dye/l blood gave a deflection of 10 cm. To conserve blood volume, the sampled blood was reinfused on completion of each dilution curve. A catheter was placed in a femoral vein for infusion of l-epinephrine bitartrate and of isoproterenol hydrochloride.

In the first series of experiments, the effects of isoproterenol on cardiac output, heart rate, stroke volume and arterial and venous pressures were measured both before and after administration of propranolol. Isoproterenol was given in successive doses of 0.05, 0.1, 0.2 and 0.4 μg/min I.V. for 3 minutes, the measurements being made on each occasion during the third minute. Propranolol was given in a single dose of 0.2 mg/kg I.V., and 20 minutes were permitted to elapse before the responses to the infusions of isoproterenol were again tested.

In the second series, the hemodynamic effects of more prolonged infusions of epinephrine in a dose of 0.5 μg/kg/min were assessed, again before and after propranolol 0.2 mg/kg I.V. In order to investigate the mechanism of the bradycardia occurring during infusions of epinephrine following the administration of propranolol, in three additional experiments the studies were carried out in dogs that had previously been treated with atropine (0.1 mg/kg).

In the third series, continuous infusions of epinephrine were again given before and after propranolol, the doses used being identical with those mentioned above. Frequent samples of blood were taken for measurement of the levels of glucose and of non-esterified fatty acids (NEFA). Blood glucose was measured in the Autoanalyzer. Plasma NEFA levels were measured by the extraction and titration method of Dole(7), using Nile Blue A as the indicator.

Results. Propranolol consistently reduced the cardiac output and heart rate and had no significant effect on stroke volume or mean arterial and venous pressures. Mean values for these measurements in 9 experiments were 3.45 l/min, 112 beats/min, 32.7 ml, 118 mm Hg and 4 mm Hg, respectively, before the drug was given, and 2.56 l/min, 81 beats/min, 32.9 ml, 119 mm Hg and 4 mm Hg 20 minutes later.

Fig. 1 shows the effects of graded infusions of isoproterenol in a dog that weighed 12.5

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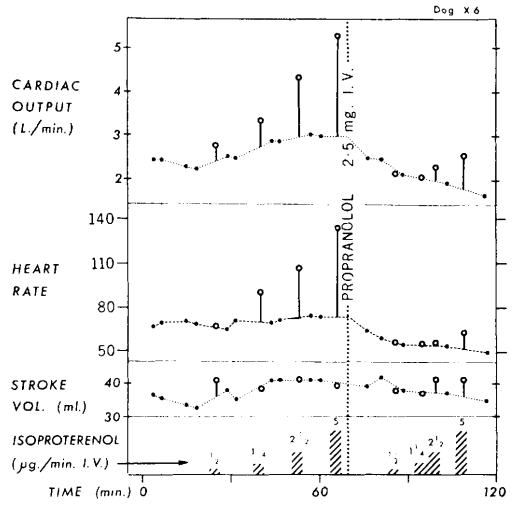

FIG. 1. Hemodynamic effects of short graded infusions of isoproterenol before and after propranolol I.V. Data obtained during control periods are shown as black dots, and successive pairs of (or single) dots are joined by interrupted lines. Data during isoproterenol infusions (cross-hatched columns) are shown as larger dots; the height of the perpendicular line connecting each such dot to the interrupted line indicates the magnitude of the response.

kg. Before propranolol, there was an evenly graded response of cardiac output and heart rate to the infusions, with no consistent change in stroke volume; after propranolol, the response to the 3 smaller infusions was abolished and that to the largest infusion was greatly reduced. Results in the other experiments were similar. For all experiments, the increase in cardiac output caused by the increasing infusion rate of isoproterenol was blocked by 107%, 96%, 85% and 60% respectively (mean values).

Fig. 2 shows the effect of epinephrine infusions on cardiac output in a dog weighing 19.8 kg. Before blockade, cardiac output was considerably increased by the infusion; after blockade, it was reduced. The reason for this is evident in Fig. 3. Before blockade, (upper panel) the arterial pressure scarcely altered during the infusion and the heart rate was also little affected apart from transitory changes during the first minute. After blockade (lower panel) the arterial pressure rapidly increased from 160/90 to 200/135 mm Hg, at which level it persisted. The sudden hypertension was accompanied by bradycardia, the heart rate suddenly slowing from 100 to 55 per minute. The slight rise in stroke volume (from 30 to 37 ml) failed to compensate for the marked bradycardia, thus accounting for the decreased cardiac output. In four other experiments, the epinephrine resulted in mean increases of 28 to 51 mm Hg in arterial pressure after propranolol and the cardiac output decreased, at least during the earlier minutes of the infusion.

It appeared probable that the bradycardia induced by epinephrine following blockade was reflex in origin, due to stimulation of the baroreceptors in the carotid sinus and aortic arch with consequent vagal slowing of the heart(8). This was tested by giving identical infusions of epinephrine before and after blocking doses of propranolol to 3 additional dogs that had previously had parasympathetic blockade induced by injections of atropine. In the atropinized dogs, the epinephrine infusions following blockade with propranolol were accompanied by sudden increases of 36, 37, and 44 mm Hg in mean arterial pressure (comparable to the increase shown in Fig. 3). However, bradycardia did not occur, and the cardiac output was either unchanged or slightly increased.

Infusions of epinephrine are normally accompanied by an increase in blood glucose and plasma NEFA levels(9). Fig. 4 shows that, immediately after an infusion of epinephrine, the glucose level was increased from a control value of 75 mg/100 ml to a peak value of 145 mg/100 ml, while plasma NEFA increased from 0.4 to 1.1 mEq/l. Propranolol blocked both of these responses. In 3 other

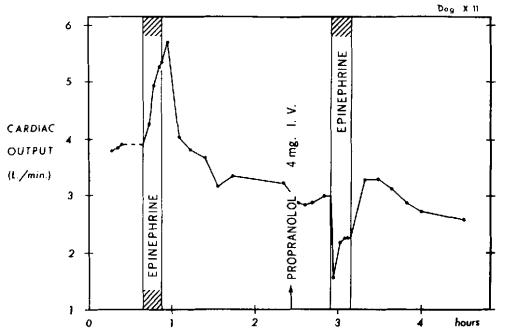

FIG. 2. Effect of 14-min infusions of epinephrine (0.5 μg/kg/min) on cardiac output before and after propranolol.

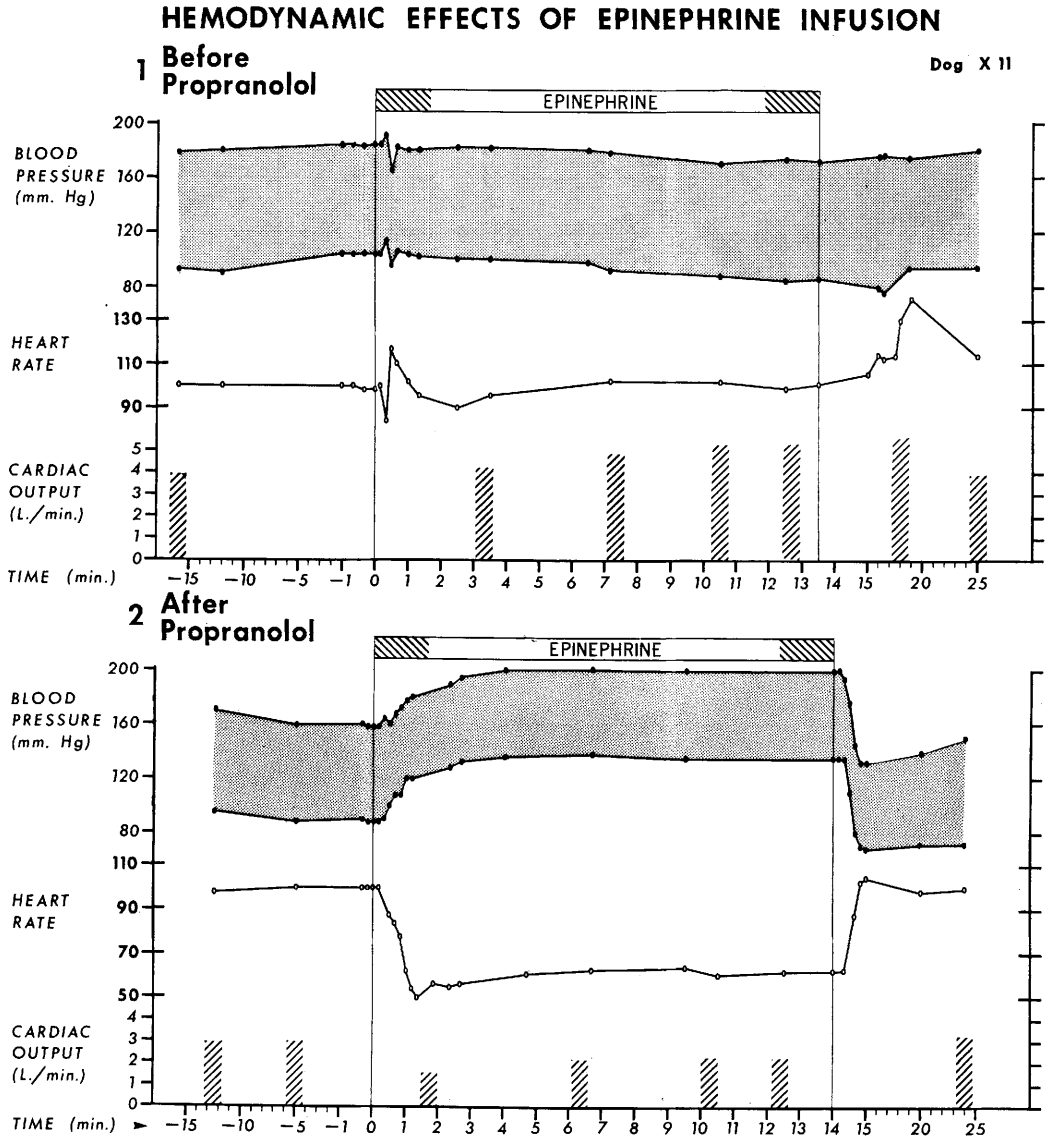

FIG. 3. Effect of 14-min infusions of epinephrine on arterial pressure and heart rate before and after propranolol.

experiments, the hyperlipidemic response to epinephrine was also completely blocked, but the hyperglycemic response was not completely blocked.

Discussion. Donald *et al*(5) showed that while pronethalol was a potent beta-adrenergic receptor blocking agent, it also consistently increased the cardiac output and stroke volume of anesthetized dogs and the heart rate of conscious dogs. They concluded that, like dichloroisoproterenol(3), pronethalol had sympathomimetic activity. In the present experiments, propranolol consistently decreased the heart rate and cardiac output of anesthetized dogs, and therefore had no demonstrable complicating sympathomimetic effect.

Propranolol (0.2 mg/kg I.V.) completely blocked the cardiovascular responses to the pure beta-adrenergic receptor agonist, isoproterenol, when this was infused at rates of 0.05 to 0.1 μg/kg/min, and reduced the effects of larger infused doses.

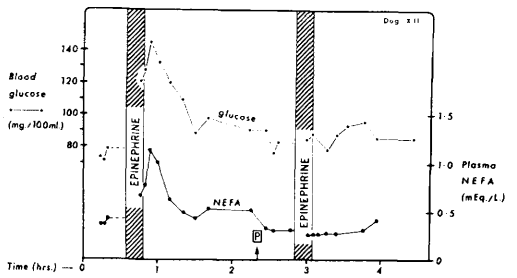

FIG. 4. Changes in blood glucose and plasma NEFA levels in response to epinephrine infusions. I.V. injection of propranolol is shown by the arrow.

Prior to blockade, epinephrine had little effect on arterial blood presure, but increased the cardiac output. Following blockade the arterial pressure was greatly increased and the heart rate and cardiac output decreased. This is explained by the fact that epinephrine is an agonist of both alpha and beta receptors. When the latter are blocked, activity at the former predominates, resulting in intense peripheral vasoconstriction, hypertension and slowing of the heart. That the slowing was reflexly induced was clear from the fact that it did not occur in atropinized dogs.

Release of NEFA from adipose tissue stores is to a large extent under the control of the sympathetic nervous system. Infusions of epinephrine(9) and norepinephrine(10) cause temporary increases in plasma NEFA levels. Most authors(11,12) found that pronethalol prevented the catecholamine-induced increase from occurring. However, Riggilo and Kyam(13) reported that, although pronethalol blocked the hyperglycemic effect of catecholamines in dogs, it failed to inhibit the increase in NEFA levels. In our studies, propranolol consistently and completely blocked the release of NEFA by epinephrine even when its hyperglycemic effect was not completely abolished.

1. Powell, C. E., Slater, I. H., J. Pharm. Exp. Ther., 1958, v122, 480.

2. Moran, N. C., Perkins, M. E., ibid., 1958, v124, 223.

3. Dresel, P. E., Canad. J. Biochem. Physiol., 1960, v38, 375.

4. Black, J. W., Stephenson, J. S., Lancet, 1960, v ii, 311.

5. Donald, D. E., Kvale, J., Shepherd, J. T., J. Pharm. Exp. Ther., 1964, v143, 344.

6. Black, J. W., Crowther, A. F., Shanks, R. G., Smith, L. H., Dornhorst, A. C., Lancet, 1964, v1, 1080.

7. Dole, V. P., J. Clin. Invest., 1956, v35, 150.

8. Heymans, C., Neil, E., Reflexogenic areas of the cardiovascular system, Little, Brown & Co., Boston, 1958.

9. Havel, R. J., Goldfien, A., J. Lipid Research, 1959, v1, 103.

10. Nestel, P. J., J. Clin. Invest., 1964, v43, 77.

11. Muir, G. G., Chamberlain, D. A., Pedoe, D. T., Lancet, 1964, v ii, 930.

12. Schröeder, G., Björntorp, P., Acta Med. Scand., 1964, v176, 395.

13. Riggolo, D. A., Kyam, D. C., Fed. Proc., 1964, v23, 124.

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Separation and Quantitative Recovery of Iodinated Amino Acids and Iodide by Thin-Layer Chromatography.* (31567)

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The physical separation of iodoamino acids has been studied in the past by means of paper and column chromatography and electrophoresis(1,2). Despite its reputation as a highly useful analytical tool in organic and biologic chemistry, few papers describing the separation of thyroid hormones by 2-dimensional TLC have appeared(3,4,5). In addition, the use of 2 solvents in the same direc-

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