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METABOLIC AND CARDIOVASCULAR EFFECTS OF ISOPRENALINE AND SALBUTAMOL IN THE DOG

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1 The intravenous infusion of isoprenaline and salbutamol into the greyhound increased heart rate and levels of free fatty acids, lactic acid and glucose.

2 On terminating the infusions of isoprenaline the changes produced declined rapidly but the effects produced by salbutamol were more persistent. When high doses of salbutamol had been infused, glucose and lactic acid levels in fact increased during the 20 min following the infusions.

3 These results support suggestions that, in the dog, lipolysis is mediated by β_1-adrenoceptors and liver glycogenolysis by β_2-adrenoceptors. The β-adrenoceptors mediating muscle glycogenolysis could not be assigned unequivocally to either subtype.

4 The differences in the behaviour of isoprenaline and salbutamol in the period following the infusions are considered to be due partly to slower removal of salbutamol. Increases in lactic acid levels after infusion of large amounts of salbutamol may be secondary to the persistence of high glucose levels.

Introduction

Previous studies indicate that liver and muscle glycogenolysis in the dog are mediated by β-adrenoceptors. McCutcheon (1962) showed that the β-adrenoceptor blocking agent, dichloroisoprenaline (DCI), inhibited catecholamine-induced rises in lactic acid and glucose levels in the dog and that the α-adrenoceptor blocking agent, phenoxybenzamine, had no effect on these responses. Mayer, Moran & Fain (1961) demonstrated that DCI inhibited the rises in fatty acid, lactic acid and glucose levels produced by infusions of adrenaline in the dog. When they proposed the subdivision of β-adrenoceptors, Lands, Arnold, McAuliff, Luduena & Brown (1967) suggested that lipolysis and cardiac stimulation were mediated by β_1-adrenoceptors and that bronchodilatation and vasodepression were mediated by β_2-adrenoceptors. Arnold, McAuliff, Colella, O'Connor & Brown (1968) examined the relative effects of isoetharine, adrenaline and noradrenaline in producing hyperglycaemia and hyperlacticacidaemia in the dog and suggested that both effects were mediated by β_2-adrenoceptors, since isoetharine was more potent than adrenaline or noradrenaline in producing them.

Salbutamol, a drug which has a greater effect on β_2- than on β_1-adrenoceptors, has been studied by Cullum, Farmer, Jack & Levy (1969) and by Ekue, Shanks & Zaidi (1971). These workers showed that in dogs, salbutamol had approximately one-eighth the potency of isoprenaline in producing increases in femoral blood flow (a β_2-adrenoceptor response) but had only one-two hundredth the potency of isoprenaline in increasing heart rate (a β_1-adrenoceptor response) but no study of the metabolic effects of this compound in the dog has been reported. Such a study is described here. The examination of salbutamol in comparison with isoprenaline was expected to elucidate further the division of the β-adrenoceptors involved in metabolic responses into β_1- and β_2-subtypes. Changes in heart rate and femoral blood flow produced by isoprenaline and salbutamol have been measured in parallel.

Methods

Observations were made in greyhounds of either sex, weighing 17-30 kg. The animals were fasted for 24 h prior to experiments. The dogs were anaesthetized with pentobarbitone (30 mg/kg i.v.) and artificially respired with room air using a Starling Ideal pump at a rate of 18 strokes/min and a stroke volume of 13 ml/kg body weight. Needle electrodes were inserted subcutaneously for recording the electrocardiogram (Lead II) and heart rate.

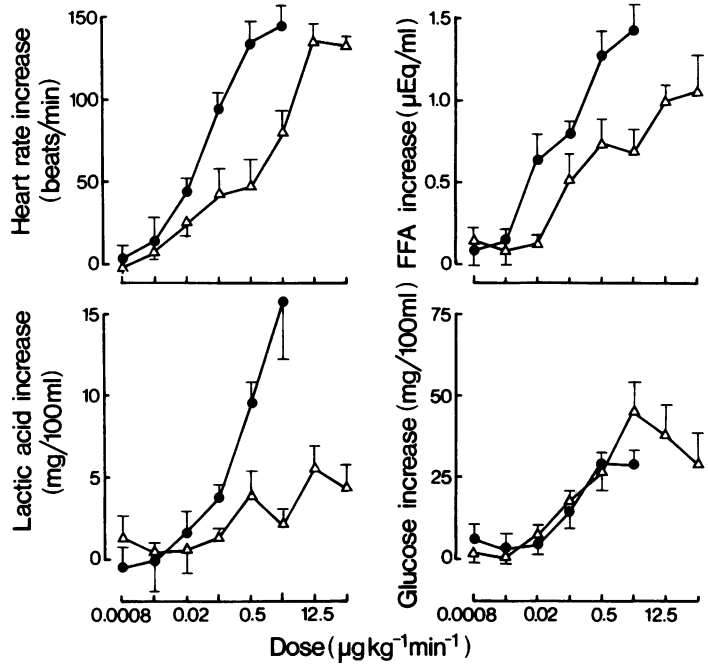

Figure 1 Mean increases in heart rate and in free fatty acids (FFA), lactic acid and glucose levels at the end of the intravenous infusion for 10 min of a series of doses of isoprenaline (•) and salbutamol (△). Each point for isoprenaline is the mean of observations in six dogs and each point for salbutamol is the mean of observations in five dogs. Vertical bars show s.e. mean.

Intravenous infusion experiments

Polyethylene catheters were inserted into each saphenous vein, one for the administration of drugs by constant intravenous infusion and the other for obtaining blood samples for the measurement of free fatty acids, lactic acid and glucose levels. Isoprenaline and salbutamol were given as constant intravenous infusions for 10 min at the following rates: isoprenaline 0.0008, 0.004, 0.02, 0.1, 0.5, 2.5 $\mu g\ kg^{-1}\ min^{-1}$ and salbutamol 0.0008, 0.004, 0.02, 0.1, 0.5, 2.5, 12.5, 62.5 $\mu g\ kg^{-1}\ min^{-1}$. Concentrations of each drug in 0.9% w/v NaCl solution (saline) were adjusted so that the required dose was given in a volume of 2 ml per minute. Blood samples were taken before and at the end of each infusion and again 20 min later. Isoprenaline infusions were repeated after 90 min when all parameters measured had returned to baseline values; three doses of isoprenaline were infused in randomized order into each dog. Isoprenaline was given to a total of 12 dogs. Since the effects of salbutamol were prolonged, two doses were given to each dog with 2 h between each infusion. Salbutamol was given to a total of 20 dogs. As the effects of salbutamol, 12.5 $\mu g\ kg^{-1}\ min^{-1}$ and 62.5 $\mu g\ kg^{-1}\ min^{-1}$ were very prolonged (>6 h), neither dose was administered as the first dose in an experiment.

Observations were made in four dogs in which glucose was infused intravenously at 20 mg $kg^{-1}\ min^{-1}$ for 20 min (in 2 ml water/minute). Blood samples were taken for estimation of glucose and lactic acid levels before an infusion and every 10 min for the next 40 minutes.

Intra-arterial infusions in the hind limb

Changes in uptake and release of free fatty acids, glucose and lactic acid in response to the administration of isoprenaline and salbutamol were estimated by the Fick principle. An external iliac artery was exposed through a lower abdominal incision and the probe of an electromagnetic flowmeter (Medicon, K 2000) was applied to the artery proximal to the origin of the deep femoral branch. Mean blood flow was recorded. A polyethylene catheter was inserted into the deep femoral artery until its tip lay in the main artery distal to the flow probe. This catheter allowed administration of drugs and sampling of blood entering the hind limb. A second catheter

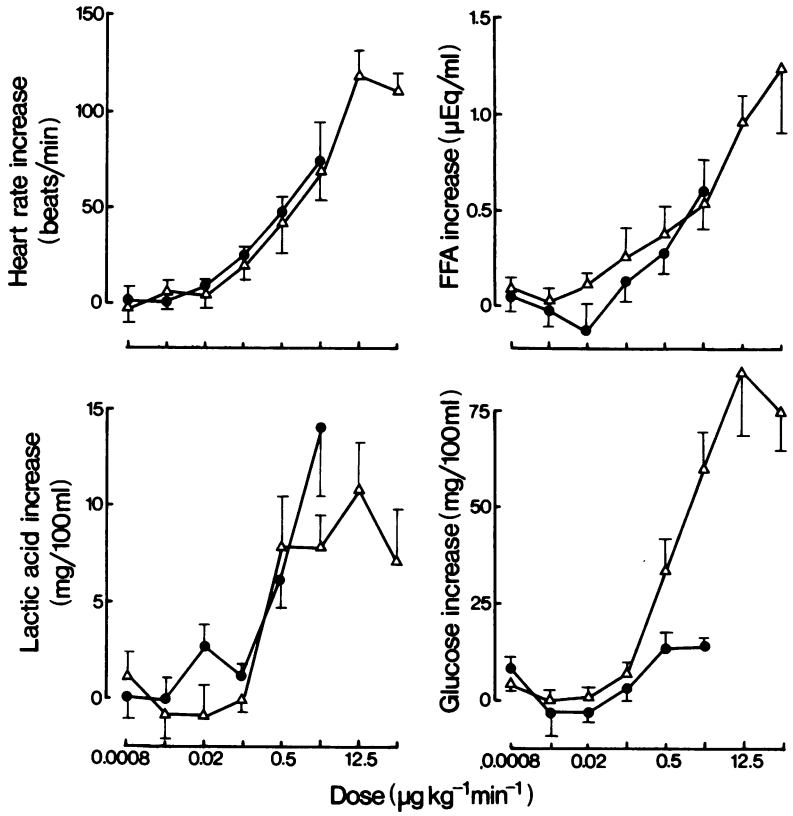

Figure 2 Mean increases in heart rate and in free fatty acids (FFA), lactic acid and glucose levels obtained 20 min after the intravenous infusion of a series of doses of isoprenaline (•) and salbutamol (△). Each point for isoprenaline is the mean of observations in six dogs and each point for salbutamol is the mean of observations in five dogs. Vertical bars show s.e. mean.

was inserted into a branch of the femoral vein to allow sampling of blood leaving the hind limb. Blood was taken from both sampling sites for estimation of free fatty acids, lactic acid and glucose before an infusion of isoprenaline or salbutamol and at the end of each infusion. Isoprenaline and salbutamol were given as 10 min infusions into the femoral artery at the following rates: isoprenaline 0.002, 0.006, 0.018 $\mu g\ kg^{-1}\ min^{-1}$ and salbutamol 0.006, 0.018, 0.054 $\mu g\ kg^{-1}\ min^{-1}$. Drugs were dissolved in saline and the concentrations were adjusted so that the required dose was given in 0.5 ml per minute.

Isoprenaline infusions were repeated after 90 min and three infusions were carried out on one day. Salbutamol infusions were repeated after 2 h and only two infusions were given to each dog. Isoprenaline was administered to five dogs and salbutamol was administered to six dogs.

Plasma concentrations of free fatty acids were estimated by the method of Mosinger (1965). Lactic acid in blood was determined by the enzymatic method of Hohorst, Krentz & Bucher (1959) and blood glucose by the method of Hultman (1959).

Drugs used were: (±) isoprenaline sulphate (Burroughs Wellcome) and (±)-salbutamol sulphate (Allen and Hanburys). All doses are expressed in terms of the base.

Results

Intravenous infusion of drugs

Mean increases in heart rate, free fatty acids, lactic acid and glucose produced by a series of increasing doses of isoprenaline in six dogs, and salbutamol in five dogs, are shown in Figure 1 in which the values obtained at the end of the period of drug administration are given.

Maximum increases in heart rate produced by isoprenaline and salbutamol were not significantly different from each other. The upper part of the

log dose-response curve for salbutamol was parallel to and to the right of that for isoprenaline but the lower part of the salbutamol curve was flatter than that for isoprenaline. Doses causing 50% of the maximum response were on the parallel parts of the curves, and their comparison showed salbutamol to have one-thirtieth the potency of isoprenaline on a weight basis.

The log dose-response curve for the effect of salbutamol on free fatty acid levels was situated to the right of that for isoprenaline and there was no significant difference between the maximum increases produced by the two drugs. The increase produced by the intravenous infusion of isoprenaline at 0.5 μg kg^{-1} min^{-1} was significantly greater ($P < 0.05$) than that produced by the same dose of salbutamol. Salbutamol produced its maximum lipolytic effects at 12.5 μg kg^{-1} min^{-1}.

The log dose-response curves for isoprenaline and salbutamol on glucose levels overlapped, no differences between the responses to the same doses of the two drugs being significant, but isoprenaline produced its maximum effects at 0.5 μg kg^{-1} min^{-1} and salbutamol at 2.5 μg kg^{-1} min^{-1}.

Isoprenaline produced dose-related increases in lactic acid levels at doses up to 2.5 μg kg^{-1} min^{-1}. Salbutamol had little effect on lactic acid levels, a significant increase ($P < 0.05$) being produced only by 12.5 μg kg^{-1} min^{-1}. Therefore a potency ratio could not be calculated.

As compared with the values found at the end of the infusion of isoprenaline (Figure 1), the values found 20 min later (Figure 2) had declined markedly in the case of heart rate, free fatty acids and glucose (all values) and slightly in the case of lactic acid ($P < 0.05$ for 0.1 and 0.5 μg kg^{-1} min^{-1} values). In contrast, at 20 min after termination of the salbutamol infusions, heart rate and free fatty acid increases were maintained. Moreover, after the higher doses of salbutamol the levels of glucose and lactic acid rose further during the 20 min period after the end of the infusion. At this time salbutamol and isoprenaline were about equipotent in their effects on heart rate, free fatty acid and lactic acid levels. The effect on glucose of salbutamol was much greater than that of isoprenaline; the slopes of the two dose-response curves differed.

Intravenous administration of glucose

Figure 3 shows the mean results of four experiments in which glucose was administered to anaesthetized dogs as an intravenous infusion at 20 mg kg^{-1} min^{-1} for 20 minutes. The blood glucose levels increased to values comparable to those found after administration of salbutamol and fell rapidly after the infusion was stopped. The lactic acid levels rose significantly ($P < 0.05$) during the infusion of glucose and were maintained during the following 20 minutes.

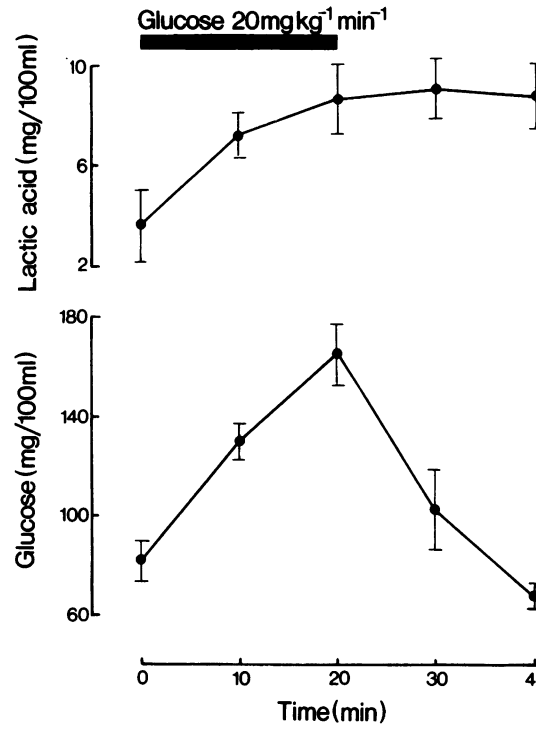

Figure 3 Mean values for lactic acid and glucose in four anaesthetized dogs in which glucose was given by intravenous infusion at 20 mg kg^{-1} min^{-1} during the period indicated. Vertical bars show s.e. mean.

Intra-arterial infusion in the hind limb

During the infusion of isoprenaline and of salbutamol into the femoral artery in anaesthetized dogs, heart rate tended to increase (not significant) and the concentrations in femoral arterial blood of free fatty acids, lactic acid and glucose did not change. Changes in femoral blood flow and in uptake or output of lactic acid found in the hind limb just after intra-arterial infusions of increasing doses of isoprenaline and salbutamol are shown in Figure 4. Isoprenaline and salbutamol had no significant effect on glucose or free fatty acid metabolism. Salbutamol did not change lactic acid levels but isoprenaline produced a dose-related increase in the output of lactic acid by the limb. Both isoprenaline and salbutamol increased blood flow to the hind limb and the salbutamol

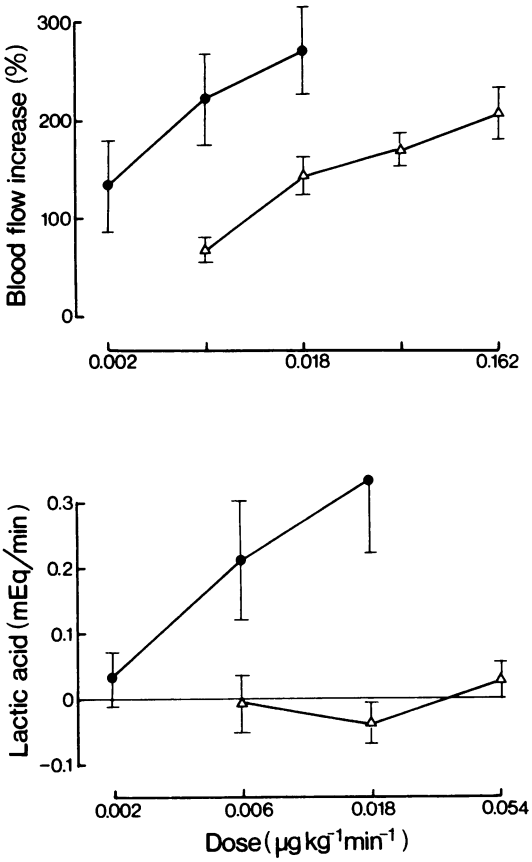

Figure 4 Mean changes in blood flow to the hind limb and output (+) or uptake (−) of lactic acid in the hind limb of anaesthetized dogs produced by the intra-arterial infusion for 10 min of a series of doses of isoprenaline (•) and salbutamol (△). Each point is the mean of five observations for isoprenaline and of four observations for salbutamol. Vertical lines show s.e. mean.

dose-response curve was to the right of the isoprenaline curve.

Discussion

The intravenous infusion of isoprenaline and salbutamol increased heart rate. Although the two log-dose response curves were of different shapes, the upper parts of each were parallel and over these dose ranges isoprenaline was 30 times more active than salbutamol. In previous studies by Ekue *et al.* (1971) in anaesthetized dogs, isoprenaline was 200 times more active than salbutamol in increasing heart rate, but in this study full dose-response curves were not examined and the drugs were administered as single intravenous doses instead of by infusion. Neither study attempted to assess the influence of reflex vagal effects produced by the drugs.

The values obtained at the termination of their intravenous infusions indicate that salbutamol is equiactive with isoprenaline in increasing blood glucose but less active in increasing heart rate, free fatty acids and lactic acid. As previous observations (Cullum *et al.*, 1969; Ekue *et al.*, 1971) have indicated that salbutamol has a greater effect on β_2- than on β_1-adrenoceptors and as the heart rate response is classified as a β_1 effect (Lands *et al.*, 1967), the present results suggest that in the dog the increase in blood glucose produced by isoprenaline and salbutamol is mediated by β_2-adrenoceptors and that the free fatty acid response is mediated through β_1-adrenoceptors. Such suggestions agree well with those of Lands *et al.* (1967) and Arnold *et al.* (1968) who suggested that in the rat, catecholamine-induced lipolysis like tachycardia was due to stimulation of β_1-adrenoceptors and that in the dog liver glycogenolysis was mediated by β_2-adrenoceptors.

Isoprenaline by close arterial infusion was more active than salbutamol in increasing blood flow to the hind leg and isoprenaline increased the output of lactic acid whereas salbutamol did not. This observation, together with the finding that salbutamol produced minimal increases in lactic acid levels at the end of intravenous infusions, indicates that muscle glycogenolysis may be mediated by β_1-adrenoceptors. In contrast Arnold *et al.* (1968) suggested that muscle glycogenolysis in the dog is mediated by β_2-adrenoceptors following studies of the maximum increases in lactic acid produced in anaesthetized dogs by the intravenous infusion of isoprenaline, noradrenaline and isoetharine.

The increased levels of heart rate, free fatty acids, glucose and lactic acid present at the end of the infusion of isoprenaline declined during the 20 min period which followed. In contrast, in this period after infusion of salbutamol, the heart rate and free fatty acid responses did not fall and when the dose was high, glucose and lactic acid levels increased. This may result at least in part from the longer half-life of salbutamol, and a delayed onset of peak metabolic change. The delayed increases in lactic acid levels after salbutamol may partially result from a conversion of glucose to lactic acid in the manner suggested by Forbath & Hetenyi (1970). That such a conversion could have occurred was shown by the increase in lactic acid produced by the infusion of glucose. The lactic acid response to isoprenaline fell much more slowly than any of the other responses to this drug

and this may also have arisen from conversion of glucose to lactic acid.

As well as comparing the effects of isoprenaline and salbutamol on some metabolic processes, this study also illustrates some difficulties encountered when examining *in vivo* subdivisions of adrenoceptors mediating metabolic responses. Difficulties occur because drugs vary in persistence and are not completely selective for one receptor subtype and because of the physiological interrelationships existing between some responses (Fleming & Kenny, 1964; Antonis, Clark, Hodge, Molony & Pilkington, 1967).

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