The effect of β-adrenoceptor blockade on the response to hypovolaemic stress in sheep

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Stress has been proposed as a major risk factor for the development of arterial disease (Carruthers, 1969). Unlike other risk factors (such as hyperlipidaemia, hyperglycaemia and hypertension) it is a difficult term to define and to measure precisely; and its role in the development of arterial disease remains controversial. Reactions to stress are considered to be primarily mediated by the secretion of catecholamines (Frankenhauser, 1971), and adrenoceptor blocking agents have been used clinically to reduce the effects of stress reactions, for example, to emotional stimuli (Imhof, Blatter & others, 1969). If catecholamines are primary mediators of stress reactions, the plasma concentrations of catecholamines or the metabolic consequences of catecholamine action may provide a useful biochemical measure of the severity of the stress. An early metabolic effect of catecholamines is to activate a membrane-bound enzyme, adenyl cyclase, in many tissues (liver, adipose tissue, skeletal muscle) to produce a second messenger, cyclic (c) AMP (Sutherland & Robison, 1966). The cyclic nucleotide then has metabolic actions like stimulating lipolysis (and raising plasma fatty acids) and activating glycogenolysis (and raising blood glucose), as well as having a direct inotropic effect on skeletal muscle. We have examined the reactions to controlled blood loss in sheep on the secretion and action of catecholamines by measuring the changes in plasma concentrations of adrenaline, and noradrenaline, and also cAMP and free fatty acids as an index of catecholamine action. We have also examined the ability of β-adrenoceptor blockade to reverse the stress reactions to hypovolaemia.

Twelve Sussex-Border crossbreed sheep (mean weight 60·5 kg) were allocated from random number tables into four groups. The animals were sedated with ketamine (20 mg kg^{-1}) to facilitate transport, immobilized on an operating table, intubated with a cuffed Magill tube and anaesthesia was induced with intravenous thiopentone (500 mg). The femoral vessels were exposed and cannulated; the vein used for sampling blood for assay of catecholamines, cAMP and free fatty acids and the artery to withdraw blood for measurements of pH, pO_2 and pCO_2. The carotid artery was cannulated to allow a Statham pressure transducer to be passed into the left ventricle for measuring blood pressure. The antecubital vein was cannulated to allow infusion of β-adrenoceptor blocking drugs (or saline in controls) by a constant volume infusion pump at 60 ml h^{-1}. Thirty min were allowed for the surgical procedures and the

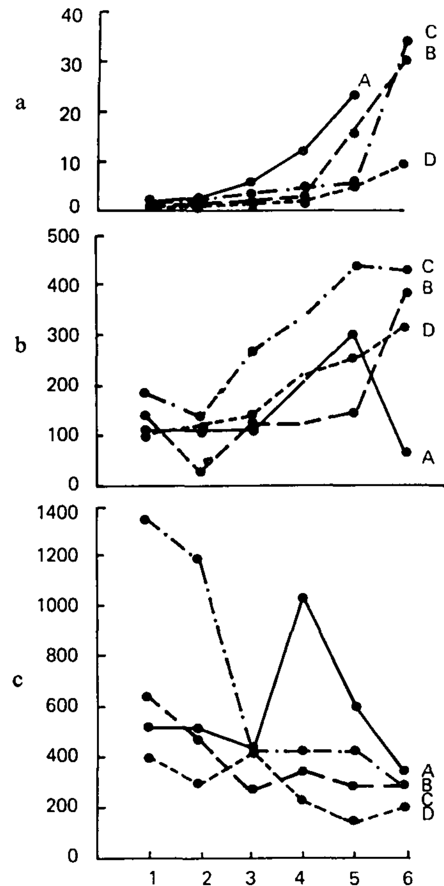

FIG. 1. The effects of graded hypotension on plasma concentrations of catecholamines, cAMP and free fatty acids in sheep treated with β-adrenoceptor blocking agents. The sheep were prepared as described in Methods and basal measurements made at periods (1) and (2) when the animals were normotensive. At periods (3), (4), (5) and (6) the systolic blood pressure was maintained at 100, 80, 60 and 40 mm Hg for 1 hour before measurements. Infusions of: A: saline control; B: propranolol (7 μg kg^{-1} min^{-1}); C: practolol (10 μg kg^{-1} min^{-1}); and D: butoxamine (200 μg kg^{-1} min^{-1}) were started after period (1). Points are means of values from three sheep. Ordinates a—Catecholamine (μg $litre^{-1}$), b—cAMP (μg $litre^{-1}$), c—Free fatty acids (μ mol $litre^{-1}$). Abscissa—Period.

effect of ketamine to disappear before starting the infusions. A resting blood specimen (sample 1, Fig. 1) was taken and called time zero. The β-adrenoceptor blocking agent (or saline in controls) was infused and a

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second sample (2) was taken 20 min later. The blood pressure was then dropped to 100 mm Hg by bleeding from the femoral artery and maintained at this level for 60 min before a blood sample (3) was obtained. Further blood samples (4, 5 and 6) were taken at 60 min intervals after the blood pressure had fallen to 80, 60 and 40 mm Hg respectively. Finally the animals were killed by halothane overdosage.

Catecholamines were assayed by placing blood in lithium heparin tubes containing sodium metabisulphate (5 mg per 10 ml) as antioxidant. The blood was spun immediately and the plasma separated for storage at −76°. Catecholamines were measured according to McCullough (1968) by a fluorometric assay with trihydroxyindole conjugation.

cAMP in plasma was assayed in samples (50–100 μl) by a competitive protein binding method, using a protein kinase with high affinity for cAMP extracted from bovine adrenal cortex according to Brown, Albano & others (1971).

Free fatty acids. These were measured in plasma by the quenching of fluorescence from sodium fluoresceinate (Carruthers & Young, 1973).

During the course of blood loss the pulse rate rose in the control group (A) over periods 1 to 5; but in sheep treated with propranolol (7 μg kg^{-1} min^{-1}; B) and practolol (10 μg kg^{-1} min^{-1}; C) the pulse rate decreased suggesting effective β-adrenoceptor blockade. The group treated with butoxamine (200 μg kg^{-1} min^{-1}; D) showed no change in heart rate; but these animals were very sensitive to small blood losses if sampling was attempted before 1 h of receptor blockade. Such blood losses resulted in uncontrolled arrhythmias, anoxia and irreversible drop in blood pressure.

The response of plasma catecholamines, cAMP and free fatty acids to hypovolaemia is shown in Fig. 1. Plasma catecholamines rose in all four groups. Plasma fatty acids rose initially in the control group (A) at samples (4) and (5) but at sample (6) had fallen to basal concentrations again; fatty acids fell to varying extents in the groups receiving β-blocking drugs. Unlike plasma fatty acids, the concentrations of cAMP rose in all groups studied.

The relation between plasma catecholamines, fatty acids and cAMP are shown in Table 1. The plasma concentrations of both adrenaline and noradrenaline correlated significantly with those of cAMP in all four groups. There was a significant positive correlation between cAMP and free fatty acids in the control group ($P < 0{\cdot}05$); but in the drug-treated group this relation was either lost (group B) or became negative (groups C and D).

The stress employed was hypovolaemia in response to graded haemorrhage. The reactions to such stress are generally considered to be due to the secretion of catecholamines which leads to tachycardia, blood vessel constriction in the skin, elevation of blood pressure and mobilization of oxidative fuels (fatty acids and glucose) by the activation of lipolysis and glycogenolysis. The main metabolic effects of catecholamines are mediated by cAMP produced by the activation of a membrane bound enzyme, adenyl cyclase, found in many tissues such as liver, adipose tissue and muscle (Butcher & Baird, 1969). Some of the circulatory manifestations of stress can be reduced by β-adrenoceptor blockade suggesting that they are in fact produced by catecholamines (Eliasch, Rosen & Scott, 1967). In our experiments tachycardia due to hypotension was abolished in animals treated with propranolol and practolol but animals treated with butoxamine showed no fall in heart rate during the stress. This partially supports the view that butoxamine has a primarily β_2-blocking effect (extracardiac) although in these animals the pulse rate did not rise as it did in untreated animals.

However, it was only possible to inhibit some of the metabolic reactions to hypovolaemic shock with β-adrenoceptor blockade. The rise in plasma fatty acids during periods of hypotension (4) and (5) was prevented in sheep treated with β-adrenoceptor blocking agents. However receptor blockade had very little effect on the rise in plasma cAMP throughout this period. The source of plasma cAMP is probably from liver (Ball, Kaminsky & others, 1972) with possible additional leakage from skeletal muscle and adipose tissue (Gilbert & Galton, 1974). Although catecholamines stimulate adenyl cyclase in these tissues, there are other hormones (such as glucagon, ACTH, TSH) which also activate adenyl cyclase in different tissues. In particular the liver is a target organ for glucagon which causes mobilization of glucose from glycogen (Exton & Park, 1968). It has also been claimed that high plasma concentrations of ACTH may not only activate adenyl cyclase in the adrenal cortex but also have extra-adrenal effects in adipose tissue (White & Engel, 1958). Although part of the metabolic reactions to stress may be mediated by cAMP such reactions are unlikely to be reduced by β-adrenoceptor blockade alone if glucagon (and possibly ACTH) are also secreted. Our results therefore do not support

Table 1. *The effects of graded hypotension on plasma concentrations of catecholamines, cAMP and free fatty acids in sheep treated with β-adrenoceptor blocking agents: a correlation matrix.*

Group treatment	Noradrenaline *vs* cAMP	Adrenaline *vs* cAMP	cAMP *vs* free fatty acid
Control (A)	r = 0·48 ($P < 0{\cdot}05$)	0·194 (N.S.)	0·63 ($P < 0{\cdot}01$)
Propranolol (B)	r = 0·66 ($P < 0{\cdot}01$)	0·74 ($P < 0{\cdot}01$)	0·001 (N.S.)
Practolol (C)	r = 0·63 ($P < 0{\cdot}01$)	0·56 ($P = 0{\cdot}01$)	−0·86 ($P < 0{\cdot}001$)
Butoxamine (D)	r = 0·83 ($P < 0{\cdot}001$)	0·71 ($P < 0{\cdot}01$)	−0·56 ($P < 0{\cdot}02$)

Correlation coefficients (r) were calculated from the data in Fig. 1 by the product-moments method; n = six pairs of observations in groups of three animals; *P* is the significance of the correlation coefficient.

the view that β-adrenoceptor blockade alone can reverse the metabolic reactions to severe stress.

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REFERENCES

BALL, J. H., KAMINSKY, N. I., HARDTMAN, J. G., BROADUS, A. E., SUTHERLAND, E. W. & LIDDLE, G. W. (1972). *J. clin. Invest.*, **51**, 2124–2129.

BROWN, B. L., ALBANO, J. D. M., EKINS, R. P., SCHERZI, A. M. & TAMPION, W. (1971). *Biochem. J.*, **121**, 561–562.

BUTCHER, R. W. & BAIRD, C. E. (1969). In: *Drugs affecting lipid metabolism.* Editor: Paoletti, R. New York: Plenum Press.

CARRUTHERS, M. E. (1969). *Lancet*, **2**, 1170–1171.

CARRUTHERS, M. E. & YOUNG, D. A. B. (1973). *Clinica chim. Acta*, **49**, 341–348.

ELIASCH, H., ROSEN A. & SCOTT, H. M. (1967). *Br. Heart J.*, **29**, 671–683.

EXTON, J. H. & PARK, C. R. (1968). *Adv. Enzyme Regul.*, **6**, 391–407.

FRANKENHAUSER, M. (1971). In: *Society, Stress and Disease*, Editor: Levi, L. Oxford: University Press.

GILBERT, C. H. & GALTON, D. J. (1974). *Horm. Metab. Res.*, **6**, 229–233.

IMHOF, P. R., BLATTER, K., FUCELLA, L. M. & TURRI, M. (1969). *J. appl. Physiol.*, **27**, 266–371.

MCCULLOUGH, H. (1968). *J. clin. Path.*, **21**, 759–763.

SUTHERLAND, E. W. & ROBISON, G. A. (1966). *Pharmac. Rev.*, **18**, 145–161.

WHITE, J. E. & ENGEL, F. L. (1958). *J. clin. Invest.*, **37**, 1556–1563.

Benzodiazepine activity: is interaction with the glycine receptor, as evidenced by displacement of strychnine binding, a useful criterion?

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Young & Snyder (1973) have clearly demonstrated that specific [^{3}H]strychnine binding to spinal cord synaptic membranes *in vitro* is associated with the glycine receptor. A correlation has also been made (Young, Zukin & Snyder, 1974) between the capacity of benzodiazepines to displace [^{3}H]strychnine binding and their potency in pharmacological and behavioural tests considered as the most relevant to clinical efficacy. The authors thus suggest that benzodiazepines may exert their anxiolytic, myorelaxant and anticonvulsant effects by mimicking the effects of glycine at its central receptor sites.

The present report is concerned with the usefulness and the specificity of the *in vitro* system for the evaluation of benzodiazepines and as a criterion for benzodiazepine-like activity.

Specifically labelled [15-^{3}H]strychnine, specific activity 11·2 Ci mmol^{-1} was prepared at Roussel-Uclaf. The labelled material behaved identically with the authentic compound on thin-layer chromatography in two different systems: on alumina (Merck, T) with benzene-ethanol (9·8 : 0·2) and on silicagel (Merck, GF 254) with chloroform–methanol–aqueous ammonia (9 : 1 : 0·2).

The preparation and incubation of synaptic membranes from rat (Sprague Dawley strain, male, 150–200 g) spinal cord and pons-medulla was carried out following essentially the method of Young & Snyder (1973). The membrane pellets, which were conserved at —28°, were resuspended in 0·05 M Na, K-phosphate buffer, pH 7·2, containing 200 mM NaCl, to give a protein concentration of 0·25–0·5 mg ml^{-1}. The suspension, in aliquots of 2 ml, was incubated in the presence of [^{3}H]strychnine (10^{-9}M) and different compounds at various concentrations for 20 min at 4°. Benzodiazepines were dissolved in ethanol so that the final concentration of ethanol in the incubation mixture was 1%. Ethanol concentrations up to at least 2·5% had no effect on specific [^{3}H]strychnine binding. The incubate was filtered on Whatman glass fibre discs, type GF/C, using a Millipore apparatus and the filters were washed rapidly (washing time less than 10 s) with 10 ml of an ice-cold solution of 0·15 M NaCl. The radioactivity on the filters was measured after digestion overnight in scintillation fluid containing 30% Triton X-100. Using the filtration technique, non-specific (NS) binding of [^{3}H]strychnine to membranes, which is defined as that not displaced by a high concentration of strychnine (10^{-4} M) or glycine (10^{-2} M), is completely eliminated. Residual binding, which amounts to about 6 % of the total on average, is independent of membrane protein concentration and is accounted for by [^{3}H]strychnine binding to the glass-fibre filters. NS binding in the centrifugation technique of Young & Snyder (1973) represents 20–30% of the total. In both techniques, however, specific binding (that which is displaced by 10^{-2} M glycine) is identical and is equal to, on average,

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