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Effect of serum albumin upon the response of the rat isolated superfused anococcygeus muscle to catecholamines and to nerve stimulation

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The presence of bovine serum albumin in the superfusing medium reduced the response of the rat isolated anococcygeus muscle to catecholamines and to nerve stimulation. It is suggested that this is a consequence of the binding of catecholamines to serum albumin. When the binding was prevented, either by decreasing the pH or by the addition of a drug such as oxytetracycline, then the inhibitory effects of serum albumin were much reduced.

There have been several reports of the ability of catecholamines to bind to serum proteins, principally to serum albumin (Antoniades, Goldfien & others, 1958; Cohen, Bralet & Rousselet, 1968; Danon & Sapira, 1972; Russell & Doty, 1973).

In the present study the effects of bovine serum albumin added to the superfusing medium, upon the responses of the rat isolated anococcygeus muscle to catecholamines and to sympathetic nerve stimulation, have been investigated.

METHODS

The binding of catecholamines to serum proteins was measured by dialysing 1 ml aliquots of bovine serum albumin 60 g litre^{-1}, or male rat serum, in sacs of Visking dialysis tubing, inflated diameter 6·4 mm (Scientific Instrument Centre), against 500 volumes of Krebs bicarbonate buffered saline pH 7·4 (Krebs & Henseleit, 1932), containing either (−)-[7-^{3}H]noradrenaline or (±)-[7-^{3}H]adrenaline, 100 ng amine base litre^{-1}, in a closed vessel at 4°. Preliminary studies revealed that 24 h was adequate for complete equilibration. Ascorbic acid, 20 mg litre^{-1}, which had no effect upon the binding, was added to retard the auto-oxidation of catecholamines.

The rat anococcygeus was chosen as an easily isolated adrenergically innervated smooth muscle preparation (Gillespie, 1972). The paired anococcygeus muscle from a male rat was isolated as described by Gillespie (1972) and superfused at a flow rate of 1 ml min^{-1} with Krebs bicarbonate buffered saline pH 7·4, containing glucose 2 g litre^{-1} at 37°. Bovine serum albumin 60 g litre^{-1} was added to the superfusate as required. The muscle was stretched to a tension of 0·75 g and the responses recorded isometrically with an electronic strain gauge. Doses of catecholamine were injected at 5 min intervals into the stream of fluid some distance before superfusing the anococcygeus, to allow for mixing. The preparation was field stimulated using bipolar platinum electrodes at a supramaximal voltage, usually, 10V, with 0·5 ms pulses for 15 s at various frequencies up to 50 Hz. A period of 3 min was allowed between successive trains of stimuli. Each preparation was superfused alternately with medium containing albumin and with albumin free medium 3 times and mean

dose-response curves constructed. A period of 15 min was allowed for equilibration when changing media.

The pH of the media was reduced below 7·4 by decreasing the concentration of $NaHCO_3$ and increased above 7·4 by decreasing the proportion of CO_2 in the gassing mixture.

Radioactively labelled catecholamines were obtained from the Radiochemical Centre, Amersham and bovine serum albumin, Fraction V powder from Pentex Research Products. Oxytetracycline HCl was kindly donated by Cyanamid.

RESULTS

Binding studies

Dialysis studies revealed that at physiological concentrations of catecholamines found in the blood, around 100 ng litre^{-1} (Engelman & Portnoy, 1970; O'Hanlon, Campuzano & Horvath, 1970), binding to male rat serum ($\pm$ s.e.) was (−)-noradrenaline 46·1 $\pm$ 1·3% (n = 10) and ($\pm$)-adrenaline 27·4 $\pm$ 1·7% (n = 10). The binding to bovine serum albumin 60 g litre^{-1} ($\pm$s.e.) was (−)-noradrenaline 42·8 $\pm$ 0·9% (n = 6) and ($\pm$)-adrenaline 22·1 $\pm$ 0·8% (n = 6).

The binding to bovine serum albumin was pH dependent; at pH 6·0 the binding ($\pm$ s.e.) of (−)-noradrenaline was reduced to 10·3 $\pm$ 1·1% (n = 6, $P < 0{\cdot}001$) and ($\pm$)-adrenaline to 7·7 $\pm$ 0·7% (n = 6, $P < 0{\cdot}001$). The tetracycline group of antibiotics were found to be inhibitors of the binding of catecholamines to bovine serum albumin. The most potent inhibitor was oxytetracycline which at 10^{-4}M reduced the binding ($\pm$ s.e.) of (−)-noradrenaline to 24·0 $\pm$ 0·7% (n = 4, $P < 0{\cdot}002$) and of ($\pm$)-adrenaline to 12·7 $\pm$ 0·9% (n = 4, $P < 0{\cdot}001$).

Responses of the rat anococcygeus

Bovine serum albumin added to the superfusate had no effect upon the passive resting tension developed by the preparation. There was, however, a reduction in the response to both noradrenaline and adrenaline (Fig. 1) and to nerve stimulation (Fig.2). The maximum response of the anococcygeus to adrenaline and to nerve stimulation was not affected by the presence of albumin. The maximum response to noradrenaline was not elicited in the dose range employed. The effects of bovine serum albumin were completely reversible.

It is unlikely that a reduction in the concentration of free Ca^{2+} as a result of its binding to albumin (McLean & Hastings, 1935) could account for the reduced response of the anococcygeus to catecholamines. Increasing the $CaCl_2$ in the medium to 10 mM had no effect upon the reduced responses caused by albumin.

Oxytetracycline 10^{-4}M, added to the superfusate, almost completely abolished the inhibitory effects of albumin. Oxytetracycline itself caused only a small potentiation in the response of the anococcygeus to catecholamines and to nerve stimulation (results not shown).

An increase in the pH of the superfusate from 6·0 to 8·0 produced a small but non-significant decrease ($P > 0{\cdot}05$) in the response of the anococcygeus to a fixed dose of noradrenaline (Fig. 3). With bovine serum albumin present in the superfusate there was a 90·0% ($P < 0{\cdot}001$) inhibition in the response as the pH increased from 6·0 to 8·0. This is probably related to the increased binding of noradrenaline to albumin at higher pH values. It is not clear, however, why the response in the presence of bovine serum albumin should be 19·4% lower ($P < 0{\cdot}02$) at pH 6·0 than at 6·5.

Fig. 1

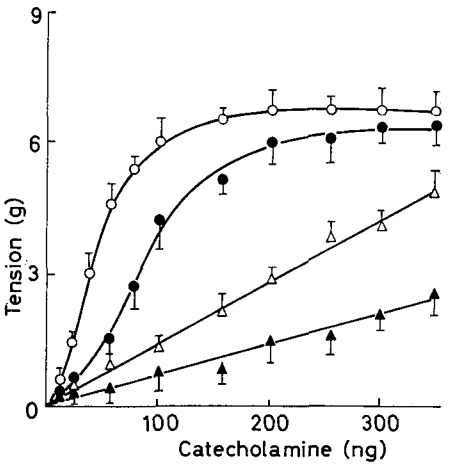

Fig. 2

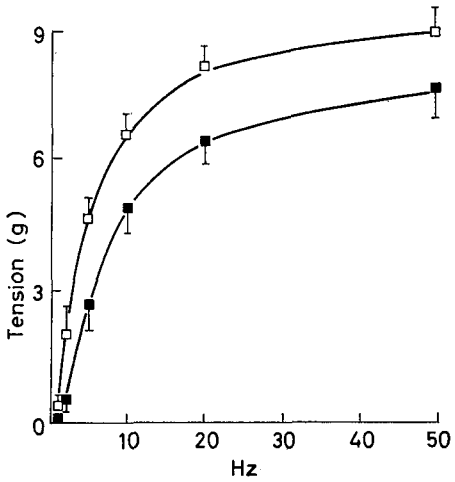

FIG. 1. Response of the rat isolated superfused anococcygeus muscle to catecholamines. Superfused at a flow of 1 ml min^{-1} with Krebs bicarbonate buffered saline (open symbols) or with Krebs bicarbonate buffered saline containing bovine serum albumin 60 g $litre^{-1}$ (closed symbols). Adrenaline (○); noradrenaline (△). Bars represent s.e. (n = 4).

FIG. 2. Response of the rat isolated superfused anococcygeus muscle to nerve stimulation. Field stimulated at a supramaximal voltage with 0·5 ms pulses for 15 s. Superfused with Krebs bicarbonate buffered saline (□) or with Krebs bicarbonate buffered saline containing bovine serum albumin 60 g $litre^{-1}$ (■). Bars represent s.e. (n = 4).

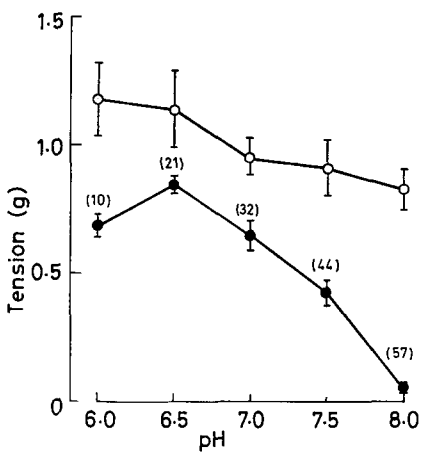

FIG. 3. Effect of pH upon the response of the isolated superfused rat anococcygeus muscle to 50 ng doses of noradrenaline. Superfused with Krebs bicarbonate buffered saline (○) or with Krebs bicarbonate buffered saline containing bovine serum albumin 60 g $litre^{-1}$ (●). Bars represent s.e. (n = 5). Figures in parentheses show the per cent noradrenaline bound as measured in dialysis studies.

DISCUSSION

The concentration of albumin used in the present study, 60 g $litre^{-1}$, is undoubtedly higher than found in serum (Sober, 1968) but was found to bind catecholamines to a similar extent as rat serum. Rat serum itself led to a progressive increase in the resting tension developed by the preparation and was unsuitable as a superfusate.

The presence of bovine serum albumin in the superfusing medium led to a decreased response by the isolated anococcygeus muscle to catecholamines and to sympathetic nerve stimulation. When the binding of catecholamines to albumin was prevented, either by decreasing the pH or by the addition of a drug such as oxytetracycline, then the inhibitory effects of serum albumin were considerably reduced. The lack of an absolute correlation between the degree of binding, as measured in dialysis studies, and the extent of the inhibition of the tissue response may be related to the difference in the temperature at which the two studies were conducted, 4° and 37° respectively. An increase in temperature is known to increase the extent of the binding of a small molecule to albumin (Karush, 1950; Opiénska Blauth, Charezinski & Brzuskkiewicz, 1963).

In both the intact animal and isolated tissues it is probable that conditions which alter the degree of binding of catecholamines to serum proteins will affect the apparent sensitivity of receptors to catecholamines. A decrease in the pH of the serum which can occur under physiological conditions (Van Slyke, 1921), but which is more likely to occur under experimental conditions, will lead to an apparent increase in the sensitivity of the receptors. Drugs which displace catecholamines from their binding sites on serum proteins will similarly lead to an increase in the apparent sensitivity of the receptors.

It is possible that albumin or other proteins present in biological samples may affect the response of preparations used for the bioassay of catecholamines and other amines. Histamine (Durand, Feger & others, 1971) and 5-hydroxytryptamine (Kerp & Kasemir, 1962) have also been reported to bind to serum proteins. Ideally, therefore, standards should be prepared in a solution with a similar protein composition to those being assayed.

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