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On the preparation of highly purified slow reacting substance of anaphylaxis (SRS-A) from biological extracts

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Ever since its discovery by Kellaway & Trethewie in 1940, Slow Reacting Substance of Anaphylaxis (SRS-A) has defied all attempts to identify it. This is partly due to the lack of suitable techniques for purification and preparation and thus an investigation of the pharmacology of this most interesting mediator has been hampered. We now present a method for the production of SRS-A of very high purity and specific activity, which is eminently suited for pharmacological investigations. Crude rat SRS-A, generated either by antigen challenge (Orange, Valentine & Austen, 1968) or by the calcium ionophore technique (Bach & Brashler, 1974) was diluted with ice-cold ethanol (1:4), the precipitated protein was removed by filtration and the ethanolic solution of SRS-A was evaporated to dryness and resuspended in distilled water. After base hydrolysis (1 N NaOH for 1 h) the aqueous mixture was pumped through a column of Amberlite XAD-8, and washed through with distilled water. The SRS-A activity was then eluted with ethanol/water (80:20, v/v). After concentration under vacuum the SRS-A was applied to a column of Sephadex G-10 equilibrated with 1% ammonium bicarbonate. Serial fractions were collected and those containing SRS-A-like activity on the guinea-pig ileum were pooled. Water and buffer salts were subsequently removed by lyophilization, and the residue was applied to a silicic acid column equilibrated in hexane. Successive elutions were made with hexane, ether and ethyl acetate and continued with increasing concentrations of methanol in chloroform. SRS-A always eluted between 40–60% methanol. Subsequent high pressure liquid chromatography (HPLC) was performed with a Waters 6060 liquid chromatograph equipped with a μBondapak-C_{18} reversed phase column equilibrated with distilled water and eluted with a linear gradient of ethanol. When first prepared SRS-A eluted as a single peak of biological activity (at an ethanol concentration of about 50%), but subsequently chromatographed as two or more active compounds of similar polarity perhaps indicating the presence of tautomers. Active fractions were pooled and further purified using the HPLC in an isocratic mode with 60% methanol–40% water as the eluting solvent. SRS-A prepared in this way has a specific activity of over 100,000 u/mg (1u $\triangleq$ 0.2 ng histamine) and is free from all other mediators.

When treated with diazomethane, SRS-A lost all biological activity but this could be (partially) restored by hydrolysis with 0.2 N NaOH at 80% for 15 minutes. This technique allows the purification of SRS-A as the less polar methyl ester and if labelled diazomethane is used, labelled methyl SRS-A may be prepared. Treatment with sodium borohydride did not inactivate SRS-A or change its chromatographic mobility. This data implies the presence of a free carboxyl, but not a ketone group on the molecule. SRS-A generated by antigen challenge or calcium ionophore was chromatographically indistinguishable.

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The effects of intra-arterial and intraportal injections of histamine on the simultaneously-perfused hepatic arterial and portal venous vascular beds of the dog

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Histamine injected into the hepatic artery of the dog causes dose-dependent hepatic arterial vasodilatation, whilst injections of histamine into the hepatic portal vein elicit dose-dependent portal vasoconstriction; both effects are mediated primarily through histamine H_1-receptors (Richardson & Withrington, 1976, 1977). We have now examined the effects of intra-arterial and intraportal injections of histamine on the sympathetically-innervated hepatic arterial and portal venous vascular beds where both were perfused simultaneously, using a combination of techniques previously reported for the separate perfusion of the two circuits: control values were similar to those in previous publications (Richardson & Withrington, 1976, 1977).

Intra-arterial injections of histamine (0.1–50 μg) elicited dose-dependent hepatic arterial vasodilatation with a maximum reduction in hepatic arterial vascular resistance (HAVR) of 44.2 ± 4.3% (mean ± s.e. mean; $n = 8$). These injections also produced dose-dependent increases in hepatic portal vascular resistance (HPVR) of up to 161.6 ± 54.5% on injection of histamine (50 μg) into the hepatic artery. The delay between the injection and onset of the hepatic arterial response to a selected dose (10 μg) of histamine (2.4 ± 0.3 s) was significantly shorter than that to the increases in HPVR (7.7 ± 1.2 s; $P < 0.01$). However, both hepatic vascular effects significantly preceded reductions of 5.0 ± 1.2 mm Hg in systemic arterial pressure (BP) resulting from histamine entering the systemic circulation (15.6 ± 1.2 s; $P < 0.005$).

Intraportal injections of histamine (0.1–100 μg) caused dose-dependent increases in HPVR (maximum = 220.5 ± 75.7%) and in addition, dose-dependent hepatic arterial vasodilatation (maximum reduction in HAVR = 37.8 ± 4.1%, $n = 8$). On intraportal injection of histamine (10 μg), the hepatic arterial response had a significantly shorter latency than the increase in HPVR (7.2 ± 0.8 s and 11.44 ± 1.3 s respectively; $P < 0.005$), and both liver vascular effects significantly preceded the onset of reductions of 6.6 ± 1.2 mm Hg in BP at 14.6 ± 1.2 s ($P < 0.02$). The lowest doses of intraportal histamine (0.1–1.0 μg) caused negligible changes in HPVR, no systemic effects, but increases in hepatic arterial blood flow of up to 15%.

The time courses of the responses to intra-arterial and intraportal histamine compared with those of the reductions in BP suggested that the effects on the two liver circuits could not be attributed to recirculation. This was further examined by injecting histamine (10 μg) into the inferior vena cava at the level of the hepatic veins: reductions of 19.5 ± 4.5 mm Hg in BP occurred 4.5 ± 1.0 s after injection, followed significantly later ($P < 0.001$) by the hepatic arterial (18.9 ± 0.9 s) and portal (16.0 ± 1.1 s) responses.

These experiments illustrate that when histamine is released into the portal bloodstream from the gastrointestinal tract in shock or anaphylaxis (Kahlson & Rosengren, 1971), it may cause increases in hepatic arterial blood flow even though vasoactive concentrations of histamine are not attained in the systemic circulation.

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