VASCULAR SMOOTH MUSCLE RESPONSE TO FIBRINOGEN DEGRADATION PRODUCTS AND 5-HYDROXYTRYPTAMINE: POSSIBLE ROLE IN CEREBRAL VASOSPASM IN MAN

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- 1 Experiments were performed to determine the effects of fibrin(ogen) degradation products on the following *in vitro* vascular preparations: rabbit aortic strip, rat aortic strip and human basilar arterial strip.
- 2 Citrated plasma and streptokinase were incubated at 37°C to produce a crude preparation of fibrinogen degradation products and 0.1 ml aliquots were removed at various time intervals. These samples were tested for intrinsic activity and possible interactions with EC_{50} and threshold concentrations of 5-hydroxytryptamine on the vascular preparations.
- 3 Enhancement of the responses obtained by both concentrations of 5-hydroxytryptamine was produced by samples taken throughout the streptokinase incubation period, the maximum effect being seen at 90 min.
- 4 The 90 min incubate produced volume-dependent (25–400 μ l) potentiations of the responses developed in the three vascular preparations to the EC_{50} and threshold concentrations of 5-hydroxytryptamine.
- 5 It is suggested that fibrin(ogen) degradation products may be involved in the intense vasoconstriction of the cerebral arteries following subarachnoid haemorrhage.

Introduction

It is generally accepted that cerebral arterial spasm following a rupture of an intracranial aneurysm is a major factor causing morbidity and mortality in humans. The intense vasoconstriction of the cerebral arteries is thought to be due to some component which exerts its effect when blood is liberated into the cerebrospinal fluid. This vasoactive substance which is most probably circulating in the cerebrospinal fluid and acting on the adventitial surfaces of cerebral vessels could be derived from the wall of the damaged vessel or from the breakdown of blood in the cerebrospinal fluid (Wilkins, Wilkins, Gunnells & Odom, 1967). A wide range of substances has been implicated in the aetiology of cerebral arterial spasm, in particular 5-hydroxytryptamine and the prostaglandins (Yamamoto, Feindel, Wolfe, Katoh & Hodge, 1972; Simeone & Vinall, 1975; Starling, Boullin, Grahame-Smith, Adams & Gye, 1975; Allen,

Gross, French & Chou, 1976). However, there is conflicting evidence regarding the functional significance of 5-hydroxytryptamine in cerebral arterial spasm (Boisvert, Weir, Overton, Reiffenstein & Grace, 1979; Boullin, Du Boulay & Rogers, 1978).

It is likely that more than one substance is involved in the development of cerebral arterial spasm and it is possible that substances which, when alone have very little effect on vascular tissue, produce marked effects when present together. Some breakdown product of the coagulation or fibrinolytic systems may also contribute to the cerebral arterial spasm. Fibrin(ogen) degradation products which have been demonstrated to be significantly elevated in cerebrospinal fluid following rupture of an intracranial aneurysm (Tovi, Nilsson & Thulin, 1973) are likely candidates. This study investigates the effect of fibrin(ogen) degradation products and their

interaction with 5-hydroxytryptamine on three *in vitro* preparations, the human basilar artery, rabbit aorta and rat aorta.

Methods

Rat and rabbit aortic strip preparation

A rat or rabbit of either sex was killed and the chest was opened. The lungs were removed and the thoracic aorta was located by removing the overlying oesophagus. A section of aorta was carefully removed and a needle was inserted through the lumen. The aorta was trimmed free of any connective tissue and kept moist with fresh, Krebs Hensleit solution bubbled with 95% O₂ and 5% CO₂. The aorta was cut in a spiral at an angle of approximately 45°, the width of the strip being approximately 3 mm. A piece of aortic strip about 3 cm long was fixed at one end of a glass tissue holder and placed in a 10 ml organ bath containing Krebs Hensleit maintained at 37°C and bubbled with 95% O₂ and 5% CO₂. The other end of the strip was secured with thread to an isometric transducer (Ether Ltd) and tension was applied and maintained on the tissue throughout the experiment (1 g for rat aortic strip and 2 g for rabbit aortic strip). The preparation was allowed to equilibrate for at least 2 h. Contractions were displayed on a Grass Polygraph recorder (Model 7D).

Human basilar arterial strip

The basilar artery was removed at post-mortem, usually between 12 and 30 h following death, from patients of either sex dying at the age of 70 years or less from non-neurological causes. A needle was inserted into the lumen of the artery and all the side vessels were removed. The tissue was then cut into a spiral strip and set up in a 10 ml organ bath as described above. A resting tension of 1.5 g was applied to the tissue, which was allowed to equilibrate for at least 2 h.

Preparation of fibrinogen degradation products

Venous blood (18 ml) from normal healthy volunteers was mixed thoroughly with 2 ml 3.8% (w/v) sodium citrate, and centrifuged at 1,000 g for 30 min at room temperature. The plasma was removed, mixed thoroughly with 2,000 units of streptokinase and incubated in a water bath at 37°C. Control samples of plasma without the addition of streptokinase were also incubated in the water bath at 37°C.

Experimental design

5-hydroxytryptamine was applied to each tissue in a cumulative manner. A maximum volume of 100 µl of the drug at each concentration was added, and the response was allowed to reach a plateau before the addition of a subsequent concentration. Then an approximate threshold concentration was chosen and the preparation was challenged repeatedly at hourly intervals with this concentration to ensure reproducibility. Similarly an approximate EC₅₀ was chosen and tested repeatedly. The approximate molar EC₅₀ and threshold concentrations of 5-hydroxytryptamine for investigation of the interaction with samples of the streptokinase incubate on the preparations were respectively 10⁻⁵ M and 10⁻⁷ M for rat and rabbit aortic strips and 2 × 10⁻⁷ M and 10⁻⁹ M for human basilar arterial strip.

Aliquots (0.1 ml) of the streptokinase incubate were removed at various time intervals up to 72 h and added to the tissue baths, followed 1 min later by either the threshold concentration of the EC₅₀ concentration without changing the bathing solution.

To determine if the enhancement of the 5-hydroxytryptamine response was dependent on the volume of incubate added, 90 min after onset of incubation the reaction was stopped by the protease inhibitor, aprotinin (Trasylo[®]) 500 units ml⁻¹ and 0.025–0.400 ml volumes of the streptokinase incubate were tested.

Drugs and solutions

The Krebs-Hensleit solution had the following composition (mM): NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0 and glucose, 11.0. Sodium citrate was BDH AnalaR grade.

Results

The concentration-effect curves to 5-hydroxytryptamine on rat aorta, rabbit aorta and human basilar artery are shown in Figure 1. It can be seen that the rabbit and rat aortae have similar sensitivities to 5-hydroxytryptamine. In contrast the human basilar artery is about 100 times more sensitive to 5-hydroxytryptamine. Aliquots (0.1 ml) of the streptokinase incubate taken at various time intervals after addition of the streptokinase were tested for intrinsic activity. Samples taken at time intervals up to 8 h (0.5, 1, 1.5, 2, 3, 4, 5 and 8 h) had no intrinsic activity on all three vascular preparations. The effect of 0.1 ml of incubate taken at 90 min tested on the human basilar artery is shown in Figure 2a. Samples (0.1 ml) of the incubate taken at time intervals of 24 and 72 h possessed intrinsic activity on all three vascular preparations. The

Figure 1 Concentration effect curves to 5-hydroxytryptamine on the human basilar artery (●), rat aortic strip (▲) and rabbit aortic strip (■). Each point represents the mean $\pm$ s.e. mean of $n=6$.

response produced was a slowly developing sustained contraction, which was easily washed out. The effect of a 0.1 ml aliquot of the streptokinase incubate taken after 24 h is shown in Figure 2b. Samples taken after 72 h produced quantitatively the same response as that produced by 24 h samples. In addition similar responses were produced on the rat and rabbit aortae preparations by the incubate. Control samples of streptokinase alone or plasma incubated without the addition of streptokinase did not possess any intrinsic activity on any of the preparations.

The ability of samples of the streptokinase incubate to interact with 5-hydroxytryptamine on the three preparations was investigated. Preliminary studies on all three preparations demonstrated that 0.1 ml samples of incubate taken at time intervals of 90 min (no intrinsic activity) and 24 h (with intrinsic activity) added to the tissue both enhanced the standard response to threshold concentrations of 5-hydroxytryptamine on all three preparations. The effect of samples taken at 90 min and 24 h on the

response of the human basilar artery to 5-hydroxytryptamine (10^{-9} M) is shown in Figure 2c and 2d respectively. Similar responses were seen on the rat and rabbit aortae. Control samples of plasma incubated without the addition of streptokinase and samples of streptokinase alone did not enhance responses to 5-hydroxytryptamine on any of the preparations used. This interaction between 5-hydroxytryptamine and the streptokinase incubate was investigated further. A fresh streptokinase incubate was prepared and left for a period of 8 h. Samples (0.1 ml) of the incubate were obtained at 0.5, 1, 1.5, 2, 3, 4, 5 and 8 h after addition of the streptokinase. In this experiment the ability of the incubate samples to enhance a standard response to 5-hydroxytryptamine (10^{-5} M) on rat aorta was studied in an attempt to determine any temporal relationship regarding this interaction. From Figure 3 it can be seen that the maximum enhancement of the 5-hydroxytryptamine response was observed with the sample taken at 90 min after onset of incubation. This time interval (90 min) was chosen to investigate the effect of various volumes of the incubate on the response of the three preparations to approximate EC_{50} and threshold concentrations of 5-hydroxytryptamine. Five volumes of streptokinase incubate were selected, 0.025, 0.05, 0.1, 0.2 and 0.4 ml. The response height to approximate EC_{50} and threshold concentrations was compared before and after the addition of the various volumes of streptokinase incubate to each of the preparations. Using a paired Student's *t*-test it was found that all the responses (except that to the threshold concentration of 5-hydroxytryptamine in the presence of 0.025 ml of streptokinase incubate on the rabbit aortic strip) to 5-hydroxytryptamine (at both concentrations) were significantly ($P<0.05$) enhanced by all volumes of streptokinase incubate used. The results are seen in Figure 4 plotted as percentage increase in the response to 5-hydroxytryptamine to increasing volumes of

Figure 2 The effect of 0.1 ml samples of the streptokinase incubate on the human basilar artery. (a) 90 min incubate sample; (b) 24 h incubate sample; (c) 90 min incubate sample added 1 min before the addition of 10^{-9} M 5-hydroxytryptamine; (d) 24 h incubate sample added 1 min before the addition of 10^{-9} M 5-hydroxytryptamine.

Figure 3 The % increase in response of the rat aortic strip to a standard concentration of 5-hydroxytryptamine (10^{-5} M) produced by pre-incubating the preparation for 1 min with 0.1 ml samples taken at various time intervals after onset of the streptokinase incubate ($n = 6$).

streptokinase incubate. The control preparations exposed to equivalent volumes of either plasma minus streptokinase or streptokinase alone had no effect on responses to 5-hydroxytryptamine on all preparations. It is apparent therefore that an incubate-volume dependent relationship exists regarding the enhancement of both EC_{50} and threshold concentrations of 5-hydroxytryptamine on all three preparations.

Discussion

This study clearly demonstrates that fibrin(ogen) degradation products have the ability not only to potentiate the action of 5-hydroxytryptamine on various vascular preparations including the human basilar artery, but in addition possess intrinsic activity on these tissues. We have also shown that the human basilar artery is much more sensitive to 5-hydroxytryptamine compared to the extracranial tissues used, rabbit and rat aorta. These factors may be of functional significance in the aetiology of cerebral arterial spasm which often results as a consequence of subarachnoid haemorrhage. Although there is no clinical evidence, it is a commonly held view that cerebral arterial spasm following subarachnoid haemorrhage can be divided into early and late phases. The early phase develops in minutes after subarachnoid haemorrhage and lasts for 1–2 days, and this is followed by the later phase which lasts for several weeks (Wilkins, 1976).

5-hydroxytryptamine is known to be released from platelets when blood coagulates (Markwardt, 1967) and therefore it is reasonable to believe that 5-hydroxytryptamine is released from platelets into the

cerebrospinal fluid after subarachnoid haemorrhage. Similarly, after subarachnoid haemorrhage it is likely that as a consequence of activation of the coagulation and fibrinolytic systems, that fibrin(ogen) degradation products will be produced in the area of the rupture and this hypothesis is consistent with the observations of Tovi *et al.* (1973) who have demonstrated that the level of fibrin(ogen) degradation products is significantly elevated in the cerebrospinal fluid after subarachnoid haemorrhage. Perhaps the most interesting observation of the present study was that aliquots of the incubate taken

Figure 4 The % increase in the response of (a) human basilar arterial strip; (b) rabbit aortic strip; and (c) rat aortic strip to approximate EC_{50} and approximate threshold concentrations of 5-hydroxytryptamine produced by various volumes of the streptokinase incubate obtained at 90 min and added to the tissue bath 1 min prior to the addition of 5-hydroxytryptamine. (●) approximate threshold concentration of 5-hydroxytryptamine. (○) approximate EC_{50} of 5-hydroxytryptamine. Each point represents the mean $\pm$ 1 s.e. mean of at least 5 results.

24 h after the addition of streptokinase possessed intrinsic activity on all the vascular preparations, an effect not seen with aliquots obtained at time intervals shorter than 24 h. This may suggest that as the incubation progresses with time different breakdown products of fibrin(ogen) are produced which have differing effects on the tissues. This would be consistent with the work of Marder, Shulman & Carroll (1969) who demonstrate a distinct sequence of breakdown of fibrin(ogen) with time into definite fibrin(ogen) degradation products which differ markedly in molecular weight. In addition, independent of the time at which the aliquot of the incubate was taken, standard responses to 5-hydroxytryptamine (near threshold and EC_{50}) were consistently and significantly enhanced. This effect appeared to be an incubate volume-dependent effect. A similar interaction has been demonstrated between fibrin(ogen) degradation products and various spasmogens including 5-hydroxytryptamine on non-vascular preparations such as the *in vitro* guinea-pig ileum and rat uterus (Buluk & Malofiejew, 1969; Malofiejew 1969).

There are, however, conflicting reports regarding the functional significance of 5-hydroxytryptamine in the aetiology of cerebral arterial spasm. Allen, Gold, Chou & French (1974) and Raynor, McMurty & Pool (1961) provide evidence to implicate 5-hydroxytryptamine in cerebral arterial spasm whereas Boisvert *et al.* (1979) and Boullin *et al.* (1978) suggest that 5-hydroxytryptamine does not play a role. The major argument stems from the observation that BW-501C67, a reputedly, relatively selective 5-hydroxytryptamine antagonist does not prevent cerebral arterial spasm in baboons induced by injection of blood intracisternally, but it did antagonize cerebral arterial spasm produced by 5-hydroxytryptamine. This is interesting since we have observed that the response of the human basilar artery to 5-hydroxytryptamine is not antagonized in a competitive manner by such 5-hydroxytryptamine

antagonists as methysergide and cyproheptadine (unpublished observations) which also appears to be the case with cat intracranial vessels (Edvinsson & Hardebo, 1978; Hardebo, Edvinsson, Owman & Svendgaard, 1978) and dog internal carotid (Saxena, 1974).

It is probable that 5-hydroxytryptamine is only one of several factors which are released during subarachnoid haemorrhage. The other major candidate is likely to be thromboxane A_2 as suggested by Boullin, Bunting, Blazo, Hunt & Moncada (1979). Recent studies have demonstrated that fibrin(ogen) degradation products can significantly enhance the actions of a wide range of prostaglandins, in particular U-46619, a stable endoperoxide analogue of prostaglandin H_2 which is thought to act via thromboxane A_2 receptors (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980) on human basilar artery (Forster, Mohan and Whalley, 1980).

In conclusion fibrin(ogen) degradation products may function as modulators of cerebral arterial spasm. In the early phase certain fibrin(ogen) degradation products have the ability to enhance the effect of other spasmogens including 5-hydroxytryptamine which are likely to be released at the time of and in the immediate period following rupture of an intracranial aneurysm. During the later phases of cerebral arterial spasm, fibrin(ogen) degradation products produced at this time may not only play a modulatory role but have a direct effect themselves on the vascular tissue involved in cerebral arterial spasm. It would therefore be of interest to determine the concentration of the different fibrin(ogen) degradation products in cerebrospinal fluid at distinct time intervals after subarachnoid haemorrhage. This is being investigated.

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