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ACTION OF SUBSTANCE P ON THE WORKING RAT HEART

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Abstract—The effect of substance P (SP) on the cardiodynamics of the isolated working rat heart perparation was examined. The peptide over the concentration range of 10^{-8} to 10^{-6} M was found to have no influence on aortic pressure, cardiac output, or cardiac work. However, a 10–15% reduction in coronary flow was observed at 1×10^{-6} M substance P. Octapeptide substance P (SP_{4-11}) exhibited a similar vasoconstrictive action. The IC_{50} of SP_{4-11} was 2×10^{-13} M compared to an IC_{50} of 3.5×10^{-8} M for substance P. Perfusion of the heart in the presence of bacitracin (1×10^{-4} M), a protease inhibitor, prevented the reduction in coronary flow observed in the presence of substance P. By contrast, the reduction in coronary flow produced by octapeptide substance P was not altered by the presence of bacitracin. Thus, it appears that a C-terminal fragment such as SP_{4-11} may be responsible for the observed decrease in coronary flow.

Substance P is an undecapeptide, whose pharmacological properties were discovered by von Euler and Gaddum [1]. However, it was not until 1970 that the peptide was purified [2], sequenced [3], and synthesized [4]. Subsequently, the synthetic undecapeptide, similar to the purified peptide, was shown to stimulate salivary secretion, to induce contraction of guinea pig ileum, and to be a potent depressor and vasodilator substance [4]. With the development of the radioimmunoassay [5] and immunohistochemical techniques [6–8] for substance P, quantitation and cellular distribution of substance P were possible.

Histochemical techniques have demonstrated the presence of substance P neurons in the heart [7–9]. Pharmacological studies have been conducted to elucidate the role of substance P neurons in regulating cardiac function [10–19]. The magnitude of the observed changes appears to be dependent upon dosage, route of administration, and species utilized. Whereas it has been reported that the action of substance P on the heart is minimal or nonexistent [10–12], other investigators have reported that substance P administration increases cardiac work [13, 14], cardiac output [13–15], heart rate [13–18], and coronary flow [19]. The coronary arterial system is also innervated by SP immunoreactive nerve fibers [7, 8]. However, the physiological function of substance P in the coronary vasculature has not been well characterized. Therefore, the effects of substance P and its analogue, octapeptide substance P (SP_{4-11}), were examined on the perfused working rat heart model. Substance P (1×10^{-6} M) produces a decrease in rat coronary flow while not altering other cardiac variables. However, substance P_{4-11}, which also altered only coronary flow, produced reductions in coronary flow at 1×10^{-12} M. It is proposed that substance P is converted to a more active fragment by a membrane-associated protease.

METHODS AND MATERIALS

Male Wistar rats (250–300 g) were utilized in all cardiac perfusions. The animals were housed in our laboratory for 3 days prior to experimentation on a 12-hr light–dark cycle and given food and water *ad lib*.

The isolated hearts were perfused, within 1 min following removal from the animal, using the working heart model of Schaffer *et al.* [20]. The perfusion apparatus consisted of two completely separate non-recirculating systems with a minimum of dead space in switching between the two buffer systems. The aorta was cannulated and perfused with buffer from a reservoir placed 100 cm above the heart. Similarly, the left atrium was attached to a cannula and received buffer from a filling reservoir maintained at a pressure of 13 cm H_2O. The hearts were paced at 300 beats/min by means of a Grass F.D. 9 stimulator. Coronary flow was determined by collecting the coronary effluent emptying into the right side of the heart. The right pulmonary artery was severed, and coronary perfusate ejected from the right pulmonary artery together with any effluent which may have escaped via the opening for the vena cave in the right atria was collected. Aortic pressure was determined by use of a Statham P23Gb pressure transducer placed in a side arm directly above the aorta, and results were recorded on a Gould 2-channel Brush 220 recorder. Cardiac work (pressure volume work) was calculated according to the equa-

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tion: C.W. = total cardiac output × mean aortic pressure × 10^{-5} [21].

The standard perfusate was Krebs–Henseleit bicarbonate buffer supplemented with 5 mM glucose and 2.5 units/l insulin. The buffer was warmed to 37° and aerated with a 95% O_2–5% CO_2 mixture to maintain pH at 7.4. Hearts were allowed to stabilize for 15 min in normal buffer prior to switching to buffer supplemented with the hormones or peptide used.

Substance P and octapeptide substance P were purchased from Penninsula Laboratories, San Carlos, CA. Bacitracin was obtained from the Sigma Chemical Co., St. Louis, MI.

RESULTS

Cardiac perfusion in the presence of substance P is shown in Table 1. These data indicate that 1×10^{-6} M substance P had no influence on aortic pressure, cardiac work, or cardiac output in the rat heart. However, SP did induce a reduction in coronary flow which was dose dependent (Fig. 1). The reduction in coronary flow was observed within 15 sec after switching from control buffer to buffer containing substance P. The threshold response was observed at 1×10^{-8} M, and a maximum decrease in coronary flow of approximately 12% was observed at 1×10^{-6} M. The IC_{50} of substance P for reduction in coronary flow, calculated from the log of the data points, was 3.5×10^{-8} M.

Octapeptide substance P was also examined for its effects on the perfused rat heart (Table 1). The octapeptide substance P had no effect on cardiac function but did produce a 12% reduction in coronary flow similar to SP (Fig. 1). However, the concentration of SP_{4-11} necessary to elicit this response was considerably lower than for SP. The IC_{50} for octapeptide substance P-induced coronary arterial constriction was 2×10^{-13} M, and the maximal effect was observed at 1×10^{-12} M.

Since the octapeptide produced a reduction in coronary flow similar to that of substance P, but at a much lower concentration, it is conceivable that substance P was subject to a membrane associated protease modification to produce the more active SP_{4-11}. Therefore, we tested the effect of the non-specific protease inhibitor, bacitracin [22, 23], in blocking the coronary vasoconstrictor action of substance P (Table 2). Perfusion of the isolated rat heart with 1×10^{-4} M bacitracin failed to show any cardiac

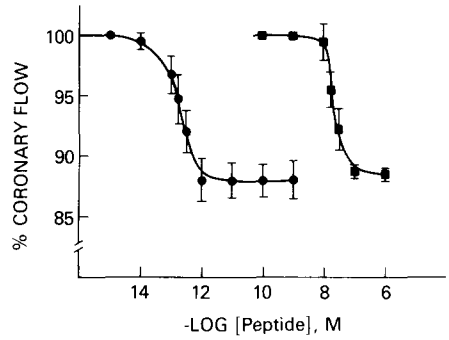

Fig. 1. Dose–response effect of substance P and octapeptide substance P on coronary flow. Hearts were perfused with appropriate concentrations of substance P (■) or octapeptide substance P (●) following an initial stabilisation period of 15 min in buffer. Coronary flow was determined after severing the right pulmonary artery, and the effluent was collected. Values reported are the means ± S.E.M. of four to six animals. The absolute value for coronary flow at 100% = 13.6 ml/min.

effects. However, this concentration of bacitracin abolished the reduction in coronary flow produced by 1×10^{-6} M SP. In contrast, the reduction in coronary flow observed with 1×10^{-11} M SP_{4-11} was unaffected by the presence of 1×10^{-4} M bacitracin in the perfusion medium.

DISCUSSION

The present findings indicate that neither SP nor SP_{4-11} appeared to regulate cardiac function in the isolated perfused rat heart. These results are consistent with those of other investigators [10–12], who demonstrated that, in the perfused guinea pig atria or papillary muscle, SP does not elicit a change in cardiac contractility. In contrast to these results, SP injections into dogs produces an increase in cardiac output, cardiac work and heart rate [13–18]. Since the tachycardia was abolished by propranolol, the effect of substance P may be related to adrenergic receptors [24]. In our study, we attempted to reduce the influence of substance P on adrenergic receptors by pacing the hearts at 300 beats/min. Under these experimental conditions, only a reduction in coronary flow was observed.

Substance P in the perfused rat heart reduced coronary flow approximately 12% at 1×10^{-6} M. Using this isolated system, SP appeared to exert its

Table 1. Effect of substance P and substance P_{4-11} on various cardiac variables*

	Concentration (M)	Aortic pressure	Cardiac output	Cardiac work	Coronary flow
Control		125.1 ± 3.6	46.0 ± 1.2	0.391 ± 0.03	13.6 ± 0.2
SP	1×10^{-6}	128.3 ± 3.5	47.5 ± 1.3	0.395 ± 0.03	12.0 ± 0.2†
SP_{4-11}	1×10^{-11}	127.8 ± 3.1	46.4 ± 1.3	0.390 ± 0.03	12.1 ± 0.1†

* Rat hearts were perfused as described in Methods and Materials. After 20-min equilibration in control buffer, the hearts were perfused with buffer containing the peptide. Values are expressed as follows: aortic pressure as cm H_2O; cardiac output as ml/min; cardiac work as kg meters · (g dry wt)$^{-1}$ · min^{-1}; and coronary flow as ml/min. Values reported are the means ± S.E.M. of four to six hearts.

† Significance of these values is $P < 0.001$.

Table 2. Effect of substance P and substance P_{4-11} on various cardiac variables in the presence of bacitracin*

	Concentration (M)	Aortic pressure	Cardiac output	Cardiac work	Coronary flow
Control		125.1 ± 3.6	46.0 ± 1.2	0.391 ± 0.03	13.6 ± 0.2
Bacitracin	1×10^{-4}	124.6 ± 3.5	45.9 ± 1.1	0.386 ± 0.03	13.6 ± 0.2
Bacitracin + substance P	1×10^{-6}	125.5 ± 4.1	44.8 ± 1.2	0.398 ± 0.03	13.9 ± 0.3†
Bacitracin + substance P_{4-11}	1×10^{-11}	127.0 ± 3.1	47.4 ± 1.4	0.390 ± 0.03	12.0 ± 0.2†

* Hearts were perfused as described in Methods and Materials. To assess the effects of bacitracin on the hearts, the hearts were equilibrated from 20 min before switching to buffer containing 1×10^{-4} M bacitracin. After an additional period of stabilisation, the hearts were perfused with the appropriate peptide. Values reported are the means ± S.E.M. of four to six hearts. Values are expressed as follows; aortic pressure as cm H_2O; cardiac output as ml/min; cardiac work as kg metres · (g dry wt)$^{-1}$ · min^{-1}; and coronary flow as ml/min.

† Significance of these values is $P < 0.001$.

vasoconstrictor action by an effect on vascular smooth muscle. Losay *et al.* [19] observed that SP in the blood perfused dog heart produced a maximal increase in coronary flow within 1 min, while Patterson* observed that, following an intitial increase in coronary flow, a marked and sustained disease in coronary flow occurred upon *in vivo* SP perfusion of canine coronary arteries. In the normal myocardium, a mild decrease in coronary flow may not be relevant as long as cardiac output is maintained. However, we may speculate that, in the stressed myocardium in which cardiac output is reduced, the magnitude of the reduction in coronary flow produced by SP may be greatly enhance and of considerable significance. Experimentation is necessary to confirm the effects of SP in the compromised myocardium.

Though substance P was found to be a potent vasoconstrictor of rat coronary arteries, the octapeptide was a more potent analogue by five orders of magnitude. These results contrast with those presented by Blumberg and Teichberg [25], who showed that SP and fragments as small as SP_{6-11} induced similar contractions of guinea pig ileum. However, Chipkin *et al.* [26], while examining the C-terminal peptides SP_{4-11} and SP_{7-11}, observed that only SP_{4-11} was more potent than SP in inducing smooth muscle contractions in both the stimulated and non-stimulated guinea pig ileum. These observations suggest that larger C-terminal fragments of SP may be at least as physiologically important as SP. There appear to be two sources for SP fragments. First, SP fragments, such as SP_{4-11}, may exist in neurons innervating vascular smooth muscle, and their release may directly alter coronary flow. The neuronal innervation of SP_{4-11} or other fragments cannot be ruled out at present since quantitative and qualitative assays for SP are cross-reactive with C-terminal fragments. However, perfusion of SP or SP_{4-11} in the presence of bacitracin, a non-specific protease inhibitor [22, 23], results in a loss of pharmacological activity of only SP. The cleavage of SP between lysine, amino acid residue 3, and proline, residue 4, results in SP_{4-11}. This proteolytic activity is similar to that observed for trypsin. However, we cannot rule out the action of other endopeptidases or aminopeptidases to produce other large active fragments of SP. Thus, based on the action of bacitracin, a membrane-associated protease located in the coronary vasculature appears to be more reasonable source for the formation of SP fragments.

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