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α-Adrenoceptor blocking action of aaptamine, a novel marine natural product, in vascular smooth muscle

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In the rabbit isolated aorta and renal artery, aaptamine (3×10^{-5} M), a novel heteroaromatic substance isolated from a sea sponge *Aaptos aaptos* produced a parallel, rightward shift of the dose-response curve for noradrenaline, whereas that for histamine or KCl was not affected. But, the derivatives of aaptamine, demethylaaptamine, demethyloxyaaptamine, dihydroaaptamine and dihydrodemethylaaptamine at concentrations of 10^{-5} to 10^{-4} M had no effect on the dose-response curve for noradrenaline. These results suggest that aaptamine is a competitive antagonist of α-adrenoceptors in vascular smooth muscles.

A number of natural products isolated from marine organisms have proved to very useful chemical tools for pharmacological, physiological and biochemical studies as they have been shown to act on specific sites of the cell membrane (Narahashi 1974; Catterall 1980; Ohizumi et al 1983; Takahashi et al 1983). In the course of our survey on pharmacologically active substances in marine organisms, much attention has been given to the occurrence of natural products possessing an α-adrenoceptor blocking activity, since these substances have played an important role in basic and clinical pharmacology (Gross 1980). Recently, a sea sponge *Aaptos aaptos* has been revealed to have a marked α-adrenoceptor blocking activity in the rabbit isolated aorta. From this sponge heteroaromatic compound named aaptamine, with a novel skeleton, 1H-benzo[de]-1,6-naphthyridine, has been isolated as an active substance (Nakamura et al 1982). The present study was undertaken to characterize the pharmacological properties of aaptamine and its derivatives using vascular smooth muscle.

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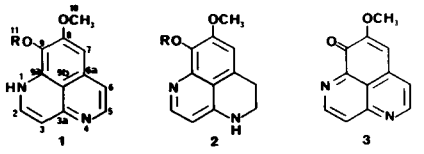
Methods

Male albino rabbits (2–3 kg) were killed by a blow on the head. The thoracic aorta and the renal artery were excised and suspended in a Krebs-Ringer-bicarbonate solution of the following composition (mM): NaCl, 120; KCl, 4·8; $CaCl_2$, 1·2; $MgSO_4$, 1·3; KH_2PO_4, 1·2; $NaHCO_3$, 25·2 and glucose, 5·8, at pH 7·4 and were aerated with a gas mixture of 95% O_2 and 5% CO_2. The method of preparing the aorta was as described by Ohizumi & Yasumoto (1983). After the connective tissue had been removed, the renal artery was cut into helical strips approximately 1 mm wide and 4 mm long. A resting tension of 1 g was applied to each strip and isometric contractions were measured by a force-displacement transducer and recorded on a polygraph.

The following drugs were used: noradrenaline bitartrate (Sigma) and histamine dihydrochloride (Wako Pure Chemical). Aaptamine hydrochloride, demethylaaptamine hydrochloride and demethyloxyaaptamine were isolated from the sea sponge *Aaptos aaptos* collected at Okinawa island and dihydroaaptamine hydrochloride and dihydrodemethylaaptamine hydrochloride were prepared as described by Nakamura et al (1982, 1983). Dihydroaaptamine hydrochloride or demethyloxyaaptamine was dissolved in ethanol to a final concentration of 0·1%. All other drugs were dissolved in distilled water as required.

Results and discussion

In both the aorta and the renal artery, NA (10^{-8} to 3×10^{-6} M), histamine (10^{-6} to 10^{-4} M) or KCl (10^{-2} to 4×10^{-2} M) caused a concentration-dependent contraction. As shown in Fig. 1, after treatment of the aorta with



1a : AAPTAMINE R=CH,
1b : DEMETHYLAAPTAMINE R=H
2a : DIH-AAPTAMINE R=CH,
2b : DIH-DEMETHYLAAPTAMINE R=H
3 : DEMETHYLOXYAAPTAMINE

aaptamine, the concentration-response curve for NA showed a parallel shift to the right, indicating competitive antagonism, whereas the histamine and KCl curves were not affected. On the basis of EC50, there was approximately a 2- and 8-fold rightward shift of the concentration-response curve for NA in the presence of aaptamine at 10^{-5} and 3×10^{-5} M, respectively. Also, in the renal artery, aaptamine at 10^{-5} and 3×10^{-5} M produced a parallel rightward shift of the concentration-response curve for NA by approximately 3- and 8-fold, respectively, without affecting that for histamine or

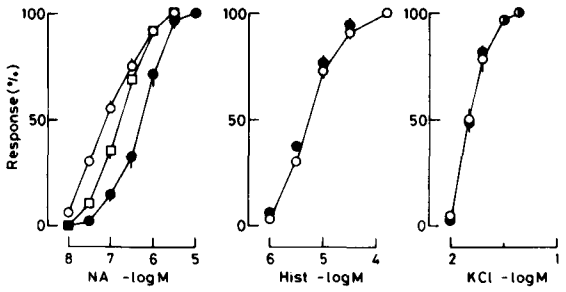


FIG. 1. Log concentration-contractile response curves for noradrenaline (NA), histamine (Hist) and KCl in the isolated rabbit aorta in the presence or absence of aaptamine. ○, control; □, aaptamine 10^{-5} M; ●, aaptamine 3×10^{-5} M. NA was cumulatively added 15 min after the application of aaptamine. The maximum response to each agonist is expressed as 100%. Symbols and vertical bars indicate means ± s.e.m. (n = 8).

KCl. The PA_2 values of aaptamine are 4·88 ± 0·16 (n = 8) in the aorta and 5·43 ± 0·09 (n = 8) in the renal artery. In addition, the PA_2 value of phentolamine on the aorta was 7·90 ± 0·04 (n = 8) under the same conditions. Washing with fresh medium 4 times at 10 s intervals abolished the inhibitory effect of aaptamine on the contractile response of each tissue to NA, indicating reversible antagonism. These data suggest that the inhibitory effect is probably specific for one agonist (NA). It is well known that in vascular smooth muscle, the NA-induced contraction is mediated through the α-adrenoceptor activation and is antagonized by specific α-adrenoceptor blocking agents (Starke & Docherty 1980). These observations suggest that aaptamine possesses α-adrenoceptor blocking activities in vascular smooth muscle. In both tissues, the inhibitory effect of aaptamine was reduced by increasing the NA concentration, and the concentration-response curve for NA showed a parallel shift to the right, suggesting that aaptamine is a competitive antagonist of α-adrenoceptors.

The contribution of functional groups in the molecule of aaptamine (1a) to the α-adrenoceptor blocking activity was studied in vascular smooth muscle. The derivatives of aaptamine, demethylaaptamine, demethyloxyaaptamine, dihydroaaptamine and dihydrodemethylaaptamine at concentrations of 3×10^{-5} to 10^{-4} M did not affect the concentration-response curve for NA, histamine or KCl in either tissue. The removal of the 11-methyl group from this compound, to give demethylaaptamine (1b), markedly decreased the activity, indicating that the existence of the methyl group is essential for the activity. Reduction of aaptamine (1a) to dihydroaaptamine (2a) also profoundly weakened the activity. In addition, a dihydrodemethyl derivative (2a) of aaptamine showed no activity. These results suggest that the aromatic property of the tricyclic ring system of aaptamine may play an important role in the development of α-adrenoceptor blocking activity.

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