The pharmacological nature of asebotoxin III-induced slower phasic contractile response to nerve stimulation in the guinea pig hypogastric nerve-vas deferens

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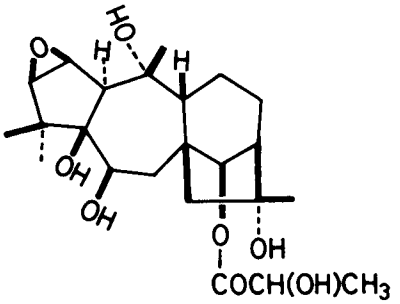
A number of diterpenic toxins have hitherto been isolated from certain ericaceous plants. One of their derivatives, α-dihydrograyanotoxin II, has been found to increase specifically sodium permeability of cytoplasmic membranes in squid axons (Seyama & Narahashi 1973). Recently, we have shown that acebotoxin III (A-III), isolated from *Pieris japonica* is the most known potent toxic substance among them (Hikino et al 1976). We have now examined the effect of A-III on adrenergic transmission using the guinea-pig hypogastric nerve-vas deferens.



The isolated hypogastric nerve-vas deferens preparation excised from a guinea-pig (250–400 g) was set up as described by Huković (1951). Selective application of drugs to either the hypogastric ganglion or the vas deferens was carried out according to Ozawa & Abe (1974). Electrical stimulation (20 Hz, 0·1 ms, 3–5 V) was applied for 4 s at 4 min intervals. Development of isometric tension in the vas deferens was recorded on a polygraph through a force displacement transducer.

At 6 min after administration of A-III (5×10^{-6}–10^{-5} g ml^{-1}) which alone gave no actions on the hypogastric nerve-vas deferens preparation, stimulation (20 Hz, 0·1 ms, 4 V, for 4 s) elicited an intense slower contraction (second contraction) following the first rapid contraction (twitch) generally caused by nerve stimulation. This response to stimulation was almost abolished at and remained so after 10 min (Fig. 1). At 15 min after administration of A-III, washing with the fresh medium did not reverse this inhibitory effect for at least 1 h. When A-III (10^{-5} g ml^{-1}) was selectively administered to the vas deferens, the biphasic contraction was also seen on stimulation (20 Hz, 0·1 ms, 4 V, for 4 s). On the other hand, when A-III (10^{-5} g ml^{-1}) was selectively applied to the ganglion, no second contraction was induced. It was thus revealed that its site of action is more peripheral than the ganglion. Consequently, A-III (10^{-5} g ml^{-1}) and all other drugs were selectively applied to the vas deferens in the following experiments. The second contraction was almost completely abolished by phentolamine (10^{-6} g ml^{-1}) or pretreatment of a guinea-pig with reserpine (0·3 mg kg^{-1} day^{-1}, for two days). Treatment with bretylium (5×10^{-7} g ml^{-1}) or tetrodotoxin (5×10^{-9} g ml^{-1}) totally abolished the second contraction, when the twitch was left almost uninfluenced. Further the second contraction was markedly potentiated by eserine (3×10^{-9} g ml^{-1}), and obliterated by hemicholinium (5×10^{-5}–10^{-4} g ml^{-1}), but was not or little affected by atropine (5×10^{-5} g ml^{-1}) or hexamethonium (10^{-4} g ml^{-1}).

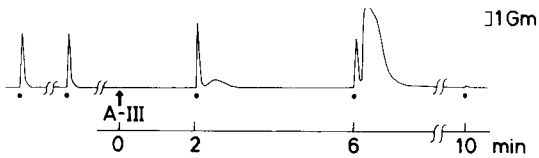


FIG. 1. A-III-induced slower phasic contraction to nerve stimulation in the isolated guinea-pig hypogastric nerve-vas deferens. Electrical nerve stimulation (20 Hz, 0·1 ms, 3–5 V) was applied for 4 s every 4 min at dots. At arrow, A-III (10^{-5} g ml^{-1}) was administered.

On the basis of the above results, it is concluded that A-III causes the release of catecholamines from the adrenergic nerve ending probably associated with the cholinergic mechanism, resulting in the second contraction.

Biphasic contraction of the vas deferens was previously reported by Swedin (1971) who showed that the mechanical response of the vas deferens to nerve stimulation (8 Hz, 1·5 ms, 12 V) for 30 s consisted of a twitch and a slower 'secondary response'. Recently, McGrath (1978) demonstrated that the 'secondary response' was always present even in response to single pulse field stimulation (0·3 ms, supramaximal voltage). It may be possible that A-III specifically exaggerates the 'secondary response' and makes it visible with the method of recording used.

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Effects of 3′,4′-dihydroxynomifensine on the dopamine vascular receptor

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Poat et al (1978) reported that 3′,4′-dihydroxynomifensine was a potent agonist of dopamine (DA) receptors in the rat striatum and nucleus accumbens, being approximately two to four times less active than DA in stimulating DA-sensitive adenylate cyclase in these brain areas. More recently, Woodruff & Sumners (1979) reported that 3′,4′-dihydroxynomifensine resembled DA in reducing blood pressure of the guinea-pig and this hypotensive action was not antagonized by propranolol, but was attenuated, like DA, by sulpiride. The present study was designed to determine the activity of 3′,4′-dihydroxynomifensine as a DA vascular agonist in the canine renal vascular bed. The effects of 3′,4′-dihydroxynomifensine on α- and β-adrenergic receptors were also studied.

All experiments were conducted in pentobarbitone-anaesthetized dogs (18–25 kg). Details of the surgical procedure and the proctocols used have been described previously (Goldberg et al 1978). In brief, renal or femoral blood flow was measured by an electromagnetic flowmeter; agonists or antagonists were injected intra-arterially, unless otherwise stated; and carotid blood pressure was recorded simultaneously with a pressure transducer.

Comparison of the effects of intra-arterial injections of DA and 3′,4′-dihydroxynomifensine on renal blood flow is shown in Fig. 1. DA and 3′,4′-dihydroxynomifensine produced dose-related increments in renal blood flow in the phenoxybenzamine-treated dog. Like DA, the vasodilation caused by 3′,4′-dihydroxynomifensine was not significantly attenuated by propranolol, 2·5 to 5 mg kg^{-1} (n = 5). This dose of propranolol was sufficient to abolish the effects of an equivasodilator dose of isoprenaline (3–12 nmol). Vasodilation by DA and 3′,4′-dihydroxynomifensine was attenuated to a similar extent (60–100%) by (±)-sulpiride in a dose of 0·5 mg intra-arterially (n = 3). In a separate experiment hexamethonium, 10 mg kg^{-1} given intravenously, abolished the effect of 25 μg kg^{-1} of 1,1-dimethyl-4-phenylpiperazinium but had no effect on renal vasodilation caused by DA or 3′,4′-dihydroxynomifensine. These experiments demonstrated that 2′,4′-dihydroxynomifensine was causing renal vasodilation by acting on DA vascular receptors.

Dose-response curves (n = 7) of increase in renal blood flow in phenoxybenzamine treated dogs produced by DA and 3′,4′-dihydroxynomifensine expressed as percent of the effect produced by 190 nmol of DA are shown in Fig. 2. The threshold dose of 3′,4′-dihydroxynomifensine was about 16-fold higher than that of DA; however, the dose-response curve of 3′,4′-dihydroxynomifensine was flatter than that of DA and the effect produced by the highest dose (12 000 nmol) of 3′,4′-dihydroxynomifensine was only 65% of the effect produced by 190 nmol of DA. Since the dose-response curves of the two compounds were not parallel, their

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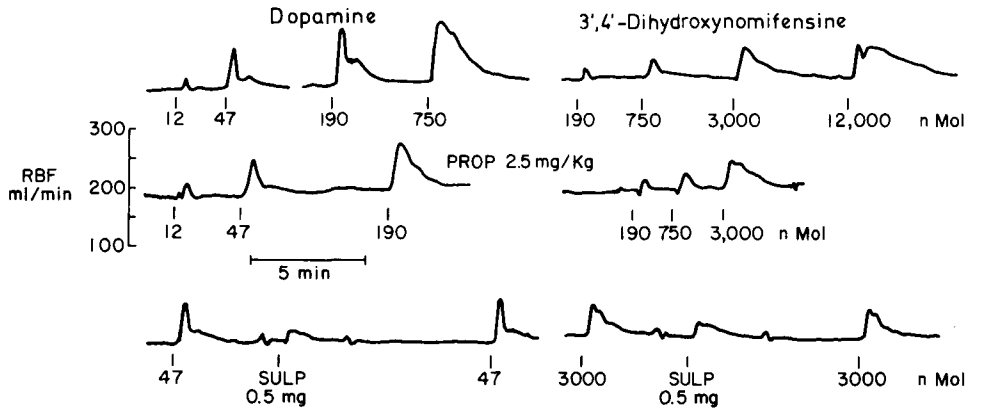

FIG. 1. Effect on renal blood flow (RBF) of increasing doses of dopamine and 3′,4′-dihydroxynomifensine injected into the renal artery in a pentobarbitone-anaesthetized dog. Top panel: after phenoxybenzamine (5 mg kg^{-1}) pretreatment, i.a. Middle panel: after subsequent administration of propranolol (Prop), 2·5 mg kg^{-1}, i.a. Bottom panel: effect of agonists alone compared with simultaneous administration of the agonist and (±) sulpiride, 0·5 mg.