EFFECT OF ACETYLCHOLINE ON RESPONSES OF THE RAT CAUDAL ARTERY

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UDC 612.133:612.181].014.46:615.217.32:
577.175.822

KEY WORDS: rat caudal artery; transmural stimulation; noradrenalin; acetylcholine; atropine.

Besides vasoconstrictor effects [8, 10], in its action of isolated vessels acetylcholine (ACh) may also evoke vasodilator responses. Depending on the site of action of ACh, two possible mechanisms of its vasodilator effect are envisaged. One is realized against the background of vasoconstriction under the influence of endogenous noradrenalin (NA), which is secreted in response to stimulation of sympathetic endings located in the vessel wall. In this case ACh evoked dilatation by acting on presynaptic endings. It activates a presynaptic muscarinic acetylcholine receptor, with consequent reduction of NA release from sympathetic endings [3, 11, 12]. On contraction of vascular segments evoked by exogenous NA or by another humoral agent, the dilator effect of ACh can also be observed. This effect is endothelially dependent and is mediated by a vasodilator factor secreted from the vascular endothelium under the influence of ACh [3-5]. This dilator response is not observed in all types of vessels and it depends on the species of animal [6]. Experiments with the endothelially-dependent dilator have been undertaken on circular preparations of large blood vessels of dogs and rabbits, a distinguishing feature of which is their poorly developed innervation.

The aim of this investigation was to study whether both types of dilator responses and interaction between them can be demonstrated on an isolated perfused vessel.

A well innervated, small vessel, the caudal artery of the rat, was chosen as the test object, and the perfusion pressure in it was recorded, so that changes in vascular resistance could be judged.

EXPERIMENTAL METHOD

The caudal artery was isolated in rats weighing 250-300 g under urethane anesthesia. A segment 6-8 mm long was excised from the proximal part of the artery, fat and connective-tissue cells were removed from it, and it was incubated for 40 min at 8-10°C in modified Krebs' solution (in mM): NaCl — 118, KCl — 4.7, $CaCl_2$ — 2.52, $MgSO_4$ — 1.64, $NaHCO_3$ — 24.88, KH_2PO_4 — 1.18, glucose 5.55, sodium pyruvate 2 (pH 7.4) [2]. The vessel was then placed in a constant-temperature chamber (37°C), aerated with carbogen (95% O_2 + 5% CO_2), and stabilized for 60 min. The segment was perfused through a metal cannula (stainless steel, external diameter 0.6 mm), on which the proximal end of the vessel was fitted. The solution escaped freely into the chamber through the distal end of the vessel. The solution was pumped through the vessel at a constant rate of 2.3 ml/min by means of a roller pump (LKB, Sweden), creating an original perfusion pressure of about 30 mm Hg in the vessel. The pressure in the vessel was determined by means of an electromanometer (Statham, USA). Transmural stimulation of the sympathetic fibers in the vessel wall was carried out with a pair of electrodes, one of which was introduced inside the vessel (it served at the same time as perfusion cannula), and the other, an open gold ring, assay 583, was fitted on the vessel from above. The parameters of ac stimulation were: 50 V, 8 Hz, 0.1 msec, 0.4-0.6 A. The time interval between successive stimulations of the preparation was 10 min. The following drugs were used: L-noradrenalin bitartrate, atropine sulfate, and phentolamine were from Sigma, USA, ACh and quinacrine were from Serva, West Germany, and guanethidine was from Egyt, Hungary. The endothelial cells

Department of Human and Animal Physiology, Faculty of Biology, M. V. Lomonosov Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR, E. I. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 3-4, July, 1986. Original article submitted July 9, 1985.

were removed by means of compressed air, which was applied under a pressure of 0.2 atm for 10 min.

EXPERIMENTAL RESULTS

The vessel was perfused with physiological saline under an average pressure for the 8 experiments of 27 ± 3 mm Hg. On injection of NA solution (10^{-7} g/ml) into the vessel a constrictor response was observed, during which the perfusion pressure rose by 56 ± 9 mm Hg ($P < 0.001$, range 95-25%, $n = 8$), and thus amounted to 83 mm Hg. Addition of ACh (10^{-6} g/ml) to the perfusion solution caused reduction of the tone created by NA by 55 ± 11% ($P < 0.02$, range 100-15%, $n = 8$). Atropine (10^{-6} g/ml) considerably reduced the effect of ACh: the very small "residual" reduction by ACh of the tone created by NA was 11 ± 2% (range 14-9%) under the influence of atropine, and was not significant ($P > 0.2$). Removal of the vascular endothelium had a similar action on the vasodilator effect of ACh. Under these circumstances the tone created by NA was not significantly changed: the increase of perfusion pressure in response to injection of NA (10^{-7} g/ml) into a vessel from which the endothelium had been removed was 50 ± 15 mm Hg ($P < 0.001$, range 25-70%). When ACh was injected into such blood vessels it reduced the tone created by NA by only 12 ± 4% (range 20-7%), which was not statistically significant ($P > 0.05$). It can accordingly be concluded that the action of ACh on a blood vessel contracted by NA depends on the presence of endothelium.

We know that the vascular tone may be created in another way (neurogenic), and that the vessel which we studied is well innervated. It might be expected that transmural stimulation of sympathetic fibers in the vessel wall would evoke constriction comparable with the action of NA and other vasoconstrictor agents. Transmural stimulation was therefore applied through two electrodes, one located inside the vessel, the other around it.

After incubation of the preparation with phentolamine (10^{-5} g/ml) for 2 min the response to NA was completely abolished, whereas that to stimulation appeared only to a pulse 0.3 msec in duration, and it increased with an increase in duration of the stimulating pulse. The concentration of phentolamine used was sufficient to block the alpha-adrenoreceptors of vascular smooth muscles, as shown by the absence of a response to NA. Hence it follows that responses to stimulation, if the stimulus duration is 0.3 msec or more, are responses to direct stimulation of the smooth-muscle cells of the vessel wall. Stimulation of shorter duration, on the other hand, is neurogenic stimulation, mediated by alpha-adrenoreceptors of smooth muscles.

ACh (10^{-5} g/ml), whether injected inside the vessel or when acting from outside, against the background of contraction evoked by transmural stimulation, caused a marked vasodilator effect. On average in 20 experiments, constriction was reduced by 69 ± 4% ($P < 0.001$). Injection of atropine (10^{-6} g/ml) completely abolished the dilator effect of ACh but did not affect the constrictor response. Where does ACh act? Two suggestions can be put forward: ACh may act through the endothelium, triggering an endothelially dependent mechanism of dilatation. The possibility likewise cannot be ruled out that it acts through presynaptic muscarinic acetylcholine receptors, activation of which reduces noradrenalin release from sympathetic endings, and this may also give rise to a considerable vasodilator response. The presence of inhibitory presynaptic muscarinic acetylcholine receptors has been demonstrated in the artery of the rabbit's ear and the pulmonary arteries, as well as in the portal and cutaneous veins [1, 7]. There are no data in the literature on the existence of presynaptic muscarinic acetylcholine receptors on sympathetic endings in the rat caudal artery, but it can be tentatively suggested that receptors of this kind do exist there. To discover which of the two possible mechanisms of the dilator action of ACh was realized in the transmural stimulation experiments, in the next experiments the endothelium was removed. Removal of the endothelium somewhat reduced the response of the preparation to stimulation but caused no significant change in the effect of ACh. These experiments were followed by others with quinacrine, which blocks endothelially-dependent vasodilatation. Quinacrine ($1.5 \cdot 10^{-3}$ g/ml) did not affect the development of the dilator effect of ACh arising against the background of stimulation of sympathetic fibers. In the first 10-15 min after addition of quinacrine, the contractile effects to NA were considerably reduced, as other workers observed previously [9]. It can be postulated on the basis of the results of the experiments with quinacrine and removal of the endothelium that in this case ACh acts on presynaptic endings by reducing NA release, which leads to vasodilatation.

On perfusion of an isolated segment of the rat caudal artery two possible mechanisms of the dilator action of ACh are thus revealed. One is exhibited in the presence of the action

of exogenous NA and is dependent on the presence of an endothelium; the other is manifested during transmural stimulation of sympathetic fibers and it is evidently realized through presynaptic inhibitor muscarinic acetylcholine receptors.

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