Perfusion-Time-Dependent Appearance of Blocking Effect of Diltiazem on Norepinephrine-Induced Vasoconstriction in Isolated, Perfused Dog Pulmonary Veins

Masayuki HANIUDA and Shigetoshi CHIBA*
Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan
Accepted October 14, 1988

Abstract—Time-dependent changes in the blocking effect of diltiazem on norepinephrine (NE)-induced vasoconstrictions were investigated in isolated canine pulmonary veins. Within 2–3 hr of the perfusion period, pretreatment with diltiazem did not affect the vasoconstrictor responses to NE. However, in the 8–11 hr perfused preparations, diltiazem suppressed the NE-induced vasoconstriction significantly. It may participate in the mechanisms for the appearance of the blocking effect in the following manner: 1) a proportion of alpha adrenoceptors which are sensitive to calcium entry are increased and/or 2) spare alpha adrenoceptors are decreased time-dependently.

It has been reported that calcium entry blocking agents reduced pressor responses to selective alpha-2 adrenoceptor agonists more effectively than the responses to selective alpha-1 adrenoceptor agonists (1, 2), with some exceptions (3). By some in vitro studies evaluating calcium influx during adrenergic stimulation (4), it was corroborated to some extent that calcium influx into smooth muscle cells was necessary for the function of alpha-2 adrenoceptors. On the other hand, it has been also suggested that the resistance of certain alpha-1 adrenoceptor-mediated pressor responses to calcium entry blocking agents is associated with the presence of a significant receptor reserve that buffers this response against inhibition by calcium entry blockers (5). Recently, we demonstrated that alpha-2 adrenoceptor mediated vasoconstrictor responses to xylazine were perfusion-time dependently enhanced in the isolated and perfused dog pulmonary veins (6). In the present study, we investigated time-dependent changes in norepinephrine-induced vasoconstriction and the blocking effect of diltiazem, bunazosin (7) or DG-5128 (8) on the NE-induced vasoconstrictions using isolated and perfused dog pulmonary veins.

Twenty-two mongrel dogs (7–16 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 units/kg, i.v.), dogs were sacrificed by rapid exsanguination from the right common carotid artery. Upon removal from the animal, the lung was placed immediately in cold Krebs-Ringer solution. After removal of loosely adhering tissues and tying branches with fine silk threads, segments of pulmonary veins (1.6–2.2 mm in outer diameter and 1.8–2.5 cm in length) were dissected carefully from the lung. Isolated pulmonary veins were cannulated as described previously (9). The isolated, cannulated vein was placed in a bath maintained at 37°C and perfused with Krebs-Ringer bicarbonate solution by means of a circulator pump (MP-3A; Tokyo Rikai Co., Tokyo, Japan). The perfusion solution was bubbled with 95% O₂ and 5% CO₂ which maintained the solution at a pH between 7.2–7.4. In addition, propranolol (10⁻⁷ M) was added to the perfusion solution to block beta-adrenoceptors. The flow rate was initially adjusted so that the perfusion...
pressure was between 5-10 mmHg, and then it was kept constant throughout the experiment (1.9-2.7 ml/min). The vasoconstrictor response was, therefore, observed as an increase in perfusion pressure.

Vascular responses to NE were observed 2 to 3 hr (early stage) and 8 to 11 hr (late stage) after setting up the perfusion preparation. Effects of antagonists (bunazosin, DG-5128 and diltiazem) on contractile response to NE were examined in both stages. These antagonists were bolusly injected 2 to 3 min before injection of NE.

Drugs used were dl-norepinephrine hydrochloride (NE, Sankyo), diltiazem hydrochloride (Tanabe), bunazosin hydrochloride (Eisai), DG-5128 (2-[2-(4,5-dihydro-1H-imidazol-2-yl)-1-phenylethyl]pyridine dihydrochloride sesquihydrate; Daiichi) and propranolol hydrochloride (ICI Pharm). All of the drug solutions, without propranolol, were administered into the rubber tube close to the cannula in a volume of 0.01-0.03 ml by use of a microinjector (Terumo Co.). The data are presented as the mean±S.E.M. in the text and illustrations.

In the early stage, NE (0.001-3 µg), which was injected into the cannulated pulmonary vein, induced dose-dependent increases in perfusion pressure, and these vasoconstrictor responses did not change statistically in the late stage. The threshold dose of NE for inducing a vasoconstriction was usually 0.001 µg, and 3 µg of NE caused a maximal increase in perfusion pressure of 15.3±1.2 mmHg (n=22). NE at a dose of 0.1 µg brought about a 50% contractile response of the maximum in both stages. In the early stage, 0.1 µg of NE induced a vasoconstriction of 10.2±1.1 mmHg (n=12). After pretreatment with increasing doses of diltiazem (1-100 µg), NE-induced vasoconstrictor responses were not affected at this stage (Fig. 1A). In the late stage, injections of NE (0.1 µg) induced vasoconstrictions (9.2±2.1 mmHg, n=12) to the same degree as in the early stage. However, at the late stage, increasing doses of diltiazem (1-100 µg) significantly suppressed the NE-induced vasoconstriction (Fig. 1B). In this stage, 100 µg of diltiazem reduced NE (0.1 µg)-induced vasoconstrictions to 58% of the control. Summarized data are shown in Fig. 2A.

In the early and late stages, effects of bunazosin (0.001-0.1 µg), a selective alpha-
1 adrenoceptor antagonist, and DG-5128 (1–100 μg), a selective α-2 adrenoceptor antagonist, on NE-induced vasoconstrictions were examined. We demonstrated previously that these doses of bunazosin and DG-5128 provided selective blockade of α-1 and α-2 adrenoceptors, respectively, in the isolated and perfused canine pulmonary vein (6). In both stages, vasoconstrictor effects of NE (0.1 μg) were antagonized by bunazosin which was injected 2–3 min before NE (Fig. 2B). In the late stage, these antagonistic effects of bunazosin on NE-induced vasoconstrictions, however, were reduced significantly. After pretreatment with increasing doses of DG-5128, vasoconstrictor effects of NE were also depressed in both stages (Fig. 2C). In contrast with bunazosin, the suppressive effects of DG-5128 on NE-induced vasoconstrictions were slightly enhanced in the late stage, but this was not statistically significant.

It has been reported that pressor responses elicited by α-1 adrenoceptor agonists are resistant to antagonism by calcium entry blocking agents, whereas responses induced by α-2 adrenoceptor agonists are sensitive to the inhibitory effects of calcium entry antagonists (1, 2). In this study, it was demonstrated that in the early and late stages, both α-1 and α-2 adrenoceptors mediated NE-induced vasoconstrictions in the isolated and perfused dog pulmonary vein, because NE-induced responses were inhibited by not only bunazosin but also DG-5128. However, the pretreatment with diltiazem, a calcium entry blocking agent, could not reduce vasoconstrictor responses to NE in the early stage. This result suggests that in the early stage, NE-induced α-1 and α-2 adrenoceptor-mediated vasoconstrictions were resistant to antagonism by diltiazem. A possible explanation for this phenomenon is that α-2 adrenoceptors which are less sensitive to calcium entry blocking agents exist in this preparation. It may be considered that there are two kinds of α-2 adrenoceptors, i.e., calcium entry sensitive and non-sensitive α-2 adrenoceptors; and in the early stage, α-2 adrenoceptors might be mostly insensitive to calcium entry in the isolated and perfused canine pulmonary veins. In 1984, Ruffolo and Yaden (10) reported that the α-1 adrenoceptor reserve was approximately 5-fold greater than the α-2 adrenoceptor reserve in the pithed rat, and the differential inhibitory effect of calcium entry antagonists may be related to differences in the number of spare receptors in the α-1 and post-junctional α-2 adrenoceptor populations (5). From the receptor reserve hypothesis, it may be also considered that in the early stage, the isolated and perfused dog pulmonary vein has enough α-1 and α-2 spare receptors to resist 100 μg of diltiazem.

In this study, it was demonstrated that there was no statistical difference in vasoconstrictor responses elicited by NE between the two stages. The blocking effect of diltiazem on NE-induced vasoconstriction, however, appeared only in the late stage. In the late stage, the suppressive effect of bunazosin on NE-induced vasoconstriction was significantly decreased. Although not statistically significant, the suppressive effect of DG-5128 on it was slightly enhanced. It seems that the proportion of NE-induced α-2 adrenoceptor-mediated vasoconstriction may be slightly enhanced by the long time perfusion. As this change is relatively small, it alone does not fully explain the time-dependent appearance of the blocking effect of diltiazem. Another possible explanation for this appearance is that the properties of α-2 and/or α-1 adrenoceptors in the isolated and perfused canine pulmonary vein might change in the case of long-time perfusion so that they become calcium influx sensitive. By the receptor reserve theory, the appearance of the blocking effect of diltiazem on NE-induced vasoconstriction may be also interpreted as indicating that the number of spare α-2 and/or α-1 adrenoceptors decrease time-dependently and can not buffer NE-induced α adrenoceptor-mediated responses against the inhibitory effects of diltiazem.

Our present results do not allow us to make conclusions about the precise mechanisms of this perfusion-time-dependent appearance of the blocking effect of diltiazem.
on NE-induced vasoconstrictions in the isolated and perfused dog pulmonary vein. Further experiments using selective alpha-1 and alpha-2 agonists, and which can demonstrate Ca++ movement directly, are necessary to provide the exact mechanism.

References