ATP-Sensitive K^+ Channels Are Gradually Recruited in the Vasodepressor Response to Adenosine in Spinally-Anesthetized Dogs

Kensuke Orito, Keisuke Satoh and Norio Taira

Department of Pharmacology, Tohoku University School of Medicine, Sendai 980, Japan

Received August 14, 1992 Accepted September 16, 1992

ABSTRACT—Vasodepressor mechanisms of adenosine were investigated in spinally-anesthetized dogs. An i.v.-infusion of adenosine (0.1 – $10 \mu\text{mol/kg/min}$) caused a slowly developing and sustained decrease in blood pressure (BP). This vasodepression was antagonized by glibenclamide, a blocker of ATP-sensitive K^+ (K_{ATP}) channels. On the other hand, a transient decrease in BP caused by a single bolus i.v.-injection of adenosine was not antagonized by glibenclamide in our previous study. These results suggested that the opening of K_{ATP} channels is gradually recruited in the vasodepressor mechanisms for adenosine-induced sustained vasodepression.

Keywords: Adenosine-induced vasodepression, ATP-sensitive K^+ channel, Glibenclamide

It has been accepted that vasodilatation or vasodepression caused by adenosine is mediated by adenosine A_2 -receptors (1, 2) and that an increase in cAMP plays an important role in this process (3, 4). Recently, Belloni and Hintze (5) suggested that opening of ATP-sensitive K^+ (K_{ATP}) channels was involved in the vasodepressor response to adenosine in anesthetized dogs; the response was antagonized by glibenclamide, a blocker of K_{ATP} channels (6). Our previous study, however, failed to demonstrate the involvement of K_{ATP} channels in the transient decrease in blood pressure (BP) caused by a single bolus i.v.-injection in spinally-anesthetized dogs; the decrease in BP caused by the adenosine injection was not antagonized by glibenclamide (7). On the other hand, the vasodepressor response to YT-146, a selective adenosine A_2 -receptor agonist (8, 9), which developed slowly and was sustained, was antagonized by glibenclamide in the same study (7). Thus, it was hypothesized that the opening of K_{ATP} channels would be gradually recruited in the vasodepressor mechanism of adenosine- A_2 -receptor-mediated vasodepression (7). To test this hypothesis we investigated how the vasodepressor response to i.v.-infusion of adenosine would be modified by glibenclamide.

Fourteen young mongrel dogs of either sex, weighing 7 to 14 kg, were anesthetized with sodium pentobarbital

(30 mg/kg, i.v.). Spinal anesthesia was induced with an intra-cisternal injection of dibucaine hydrochloride solution (about 0.2 ml/kg) after i.v.-injection of nadolol ($100 \mu\text{g/kg}$) and atropine (1 mg/kg). Bilateral vagotomy was performed. Animals were respired with room air in a tidal volume of 20 ml/kg at 18 breaths/min by the use of a respirator (Shinano, Model SN-480-3). Body temperature was maintained at about 39°C with a heating device. BP was measured at the right femoral artery with a pressure transducer (Statham, P-50) and heart rate (HR) was measured with a cardi tachometer (San-ei, 1321) triggered by arterial pressure pulses.

The BP of spinally-anesthetized animals was maintained at an elevated level by i.v.-infusion of noradrenaline ($0.5 \mu\text{g/kg/min}$) by the use of an infusion pump (Harvard Apparatus, Model 600-00). Five animals were given the vehicle (100% *N,N*-dimethylformamide, control), another five glibenclamide, and an additional four glibenclamide plus theophylline. Before receiving adenosine infusion, all the animals were given dipyridamole ($100 \mu\text{g/kg}$, i.v.), a potentiator of adenosine (10). Otherwise, i.v.-infusions of adenosine failed to produce sizable decreases in BP. An infusion pump (Terumo, STC-521) was used for the continuous i.v.-infusion of adenosine. Infusion rates were increased when the BP responses reached a nadir.

Glibenclamide (Yamanouchi), nadolol (Squibb), adeno-

sine (Sigma), theophylline (Sigma), (–)-noradrenaline base (Sigma), dibucaine hydrochloride (0.3% hyperbaric solution in ampoules, Teikoku Chemical) and atropine sulfate (Wako) were used. Glibenclamide was dissolved in 0.5 ml of 100% *N,N*-dimethylformamide at the desired concentrations. Nadolol and (–)-noradrenaline base were dissolved in 0.1 N HCl. Other compounds were dissolved and diluted with 0.9% saline to the desired concentrations.

Changes in MBP were expressed as percentages of its basal values obtained before infusion of adenosine. ED_{50} values for adenosine were obtained by computer-fitting of the dose-response curves for the change in MBP to a sigmoid function by a non-linear least squares routine. The statistical significance of differences between ED_{50} values was analyzed with Student's *t*-test. A *P* value smaller than 0.05 was considered to indicate a significant difference.

The average values of MBP and HR for fourteen spinally-anesthetized dogs, whose MBPs were maintained at an elevated level with i.v.-infusion of noradrenaline, were 176 ± 4 mmHg and 149 ± 6 beats/min, respectively. Glibenclamide or theophylline per se had no effect on MBP and HR.

In control dogs, infusion of adenosine (0.1–10 μ mol/kg/min) caused slowly developing decreases in BP and HR, and MBP was lowered to about 46 mmHg and HR to 116 beats/min at the highest infusion rate (10 μ mol/kg/min). One of such experiments is shown in Fig. 1, and the dose-response curve for the decrease in MBP is shown in Fig. 2. In dogs that received

glibenclamide (6 μ mol/kg, i.v.), the dose-response curve for the decrease in MBP underwent a rightward shift (Fig. 2); the ED_{50} value increased about 6-fold (1.3 to 7.5 μ mol/kg/min). A further rightward shift of the dose-response curve for the decrease in MBP occurred in dogs that received glibenclamide (6 μ mol/kg, i.v.) plus theophylline (30 μ mol/kg, i.v.) (Fig. 2); the ED_{50} value increased about 33-fold (1.3 to 42.6 μ mol/kg/min). Decreases in HR to adenosine infusion remained virtually unchanged by glibenclamide or glibenclamide plus theophylline.

The slowly developing and sustained vasodepressor responses to i.v.-infusions of adenosine described above were very similar to those caused by a single bolus i.v.-injection of YT-146 in time course and in being antagonized by 6 μ mol/kg, i.v. of glibenclamide (7). In the previous study (7), the transient vasodepressor responses to single bolus i.v.-injections of adenosine were not antagonized by the same dose of glibenclamide. Thus, as hypothesized previously (7), the vasodepressor mechanisms for adenosine- A_2 -receptor-mediated vasodepression likely differ between the transient response and the slowly developing response. At least in the dog, the opening of K_{ATP} channels appears to be gradually recruited to the vasodepressor mechanisms.

The approximately 6-fold increase in ED_{50} value for adenosine caused by glibenclamide in the present experiments was very close to the 5.5-fold increase in ED_{50} value for YT-146 under similar conditions in the previous study (7). However, the approximately 33-fold increase in ED_{50} value of adenosine caused by

Fig. 1. Effects of i.v.-infusions of adenosine on instantaneous blood pressure (BP), mean BP (MBP) and heart rate (HR).

Fig. 2. Dose-response curves for % change in MBP to i.v.-infusions of adenosine and their modifications by glibenclamide or glibenclamide plus theophylline. Data points represent the mean $\pm$ S.E.M. (○) Control ($n = 5$). (●) Dogs given glibenclamide ($6 \mu\text{mol/kg}$, i.v.) ($n = 5$). (▲) Dogs given glibenclamide plus theophylline ($30 \mu\text{mol/kg}$, i.v.) ($n = 4$).

glibenclamide plus theophylline was much greater than the 6.8-fold increase in ED_{50} value of YT-146 that had occurred under similar conditions in the previous study (7). This suggests that the vasodepressor response to i.v.-infusion of adenosine, which is nonselective for adenosine A_1 and A_2 receptors, may involve more complicated vasodepressor mechanisms than that to the selective adenosine A_2 -receptor agonist YT-146. Adenosine has been shown to exert its vasodilator effect by stimulation of A_1 -receptors, which are linked to guanylate cyclase (11), in addition to an action via adenosine A_2 -receptors.

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