
**Presynaptic Inhibitory Action of Adenosine on Neuromuscular Transmission in the Canine Cavernous Carotid Artery**


**SUMMARY** We investigated the effect of adenosine on neurogenic contraction of the canine cavernous carotid artery, using an isometric tension recording device and transmural nerve stimulation. Adenosine, in concentrations under $10^{-6}$ M, had no relaxing effect on the contractions produced by high (KCl) solution or $10^{-5}$ M norepinephrine. Transmural nerve stimulation (stimulus: 1 msec duration, 100 V intensity) evoked a frequency-dependent contraction, which was abolished by $3 \times 10^{-4}$ M tetrodotoxin. Adenosine in concentrations of $10^{-5}$ M and $10^{-6}$ M, inhibited the neurogenic contraction at each frequency, more so in the low frequency range. This inhibitory effect of adenosine was significantly antagonized by $10^{-5}$ M theophylline. Pretreatment with $2 \times 10^{-5}$ M dipyridamole had no effect on neurogenic contractions, but augmented the inhibitory effect of adenosine. $10^{-5}$ M theophylline did not augment the neurogenic contractions. The findings that both dipyridamole and theophylline failed to affect the neurogenic contractions in the absence of adenosine suggests that the presynaptic autoinhibition mechanism of adenosine may not be involved in neuromuscular transmission in this tissue. These results suggest that there is a presynaptic adenosine receptor in the nerve terminal which inhibits the release of neurotransmitter in canine cavernous carotid artery. It is also probable that the vasodilating effect of adenosine in the cavernous carotid artery is mainly due to its inhibitory effect on neurotransmission rather than to a direct relaxing effect on smooth muscle.

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ADENOSINE can markedly affect the blood flow in the cerebral vascular bed as well as in various peripheral vascular beds. It is well known that the vasodilating action of adenosine is due not only to a direct inhibitory action on vascular smooth muscle, but also to presynaptic inhibition of adrenergic transmission in peripheral vascular beds. However, there has been no extensive investigation into the vasodilating action of adenosine on the internal carotid artery system.

Recently, a great deal of evidence has been accumulated that the internal carotid artery system, as well as small pial vessels, play an active role in the regulation of cerebral blood flow, and an abundant efferent inner-vation by both adrenergic and cholinergic nerve fibers was demonstrated in the cavernous carotid artery (CCA). Furthermore, when we recorded the neurogenic contraction of canine CCA, the amplitude of these contractions was much larger than that of the basilar artery, and we found that the neuromuscular transmission mechanism of canine CCA was different from that of peripheral arteries (Fujiwara, S, unpublished data).

In this experiment, we investigated the effect of adenosine on canine CCA, especially concerning its effect on neurogenic contraction, in order to clarify the presynaptic role of adenosine and to discern the precise...
mechanism of the vasodilating action of adenosine on CCA.

Methods and Materials

Adult mongrel dogs of either sex, weighing 12-18 kg, were anesthetized with sodium pentobarbital (30 mg/kg) and sacrificed by exsanguination from the femoral artery. The brain was removed, and the cavernous carotid arteries were carefully dissected after opening the cavernous sinus. The specimens were immediately placed in Krebs’ solution at 25°C.

Mechanical responses were recorded by the following method: the tissue was mounted in an organ bath with a capacity of about 4 ml and superfused with warmed Krebs’ solution (35°C) at a flow rate of about 5 ml/min. A pair of L-shaped stainless steel rods, which were sharpened by electrolysis, and were positioned at opposite points inside the circumference of the ring segment of artery about 1 mm in width. One of the rods was fixed to a block at the bottom of the organ bath and the other was connected to a tension recorder (Grass FT.03). To evoke contractions by transmural nerve stimulation, a pair of silver plates fixed at both sides of the tissue were used, and a stimulus of 100V, 1 msec duration (Grass.S88) was applied.

Modified Krebs’ solution, formulated as follows, served as the control solution (mM): NaCl 120; KCl 4.5; MgSO4 1.0; NaHCO3 27.0; KH2PO4 1.0; CaCl2 2.5; and dextrose 10.0. The solution was bubbled with 95% O2 and 5% CO2. The pH was kept at 7.2-7.4. High potassium solution, 39.2 mM was prepared by isotonically replacing NaCl with an equivalent amount of KCl. To prevent the effect of high [K+]o-induced release of transmitter, 10^{-7} M guanethidine and 3 \times 10^{-7} M tetrodotoxin were added in the experiments using 39.2 mM [K+]o-induced contraction.

The following drugs were used in experiments: tetrodotoxin (TTX), norepinephrine hydrochloride (NE), adenosine, theophylline [Sigma] and dipyridamole [Boehringer]. NE was diluted with 0.1N HCl and 0.1% ascorbic acid. The solutions were freshly prepared just prior to each experiment.

Dipyridamole was dissolved in 0.5% polyethylene glycol (PEG). We checked the effect of PEG on contractions induced by 39.2 mM [K+]o and 10^{-5} M NE, as well as nerve-mediated contractions. Concentrations of PEG under 0.1% had no effect on any of the contractions described above. The concentration of PEG in the dipyridamole solution was under 0.0005%.

Obtained values were expressed as the mean ± S.D. Statistical comparison between responses of rings from the same artery in the presence and absence of adenosine was determined using Student’s t-test in figures 1, 2 and 3. In figures 4 and 5, we compared the responses between the theophylline or dipyridamole treated group and non-treated group using Student’s t-test. Probabilities of less than 5% (p < 0.05) were considered to be significant.

Results

Effect of Adenosine on Contractions of CCA Induced by 39.2 mM [K+]o Solution and 10^{-5} M NE

Both 39.2 mM [K+]o solution and 10^{-5} M NE produced contractions of the CCA, but the 10^{-5} M NE induced contractions were much smaller than those induced by 39.2 mM [K+]o when the responses of peripheral arteries were compared. The average amplitude of 10^{-5} M NE-induced contractions was 0.13 ± 0.01 (n = 8) times the 39.2 mM [K+]o-induced contractions in mesenteric artery (Fujiwara, S unpublished data).

To investigate the effect of adenosine on contractions induced by 39.2 mM [K+]o and 10^{-5} M NE, concentrations of adenosine ranging from 10^{-6} M to 10^{-3} M were cumulatively applied to sustained contractions evoked by each stimulus.

Figure 1A shows the effect of adenosine at various concentrations on 39.2 mM [K+]o-induced contractions. The amplitude of these contractions in the absence of adenosine was normalized as 1.0. Concentrations of adenosine under 10^{-5} M had no relaxing effect on 39.2 mM [K+]o-induced contractions (10^{-3} M aden- osine; 1.0 ± 0.01, n = 9; 10^{-5} M adenosine: 0.99 ± 0.02, n = 9). Concentrations over 10^{-5} M relaxed the contraction (10^{-3} M adenosine: 0.91 ± 0.05, n = 9, p < 0.05; 10^{-2} M adenosine: 0.61 ± 0.06, n = 9, p < 0.01). These inhibitory actions of adenosine on contractions induced by 39.2 mM [K+]o were not affected by 10^{-5} M theophylline.

Figure 1B shows the effect of adenosine at various concentrations on 10^{-3} M NE-induced contractions. The amplitude of 10^{-5} M NE-induced contractions in the absence of adenosine was registered as 1.0. Adenos-
sine had no relaxing effect on 10^{-3}M NE-induced contraction at concentrations under 10^{-4}M (10^{-4}M adenosine: 1.0 ± 0.02, n = 8; 10^{-3}M adenosine: 0.51 ± 0.12, n = 8, p < 0.01).

**Effect of Adenosine on Contractions Evoked by Electrical Stimulation of CCA**

Electrical stimulation evoked contractions of the CCA. To compare the effect of adenosine on contractions induced by nerve stimulation with those induced by direct muscle stimulation, we used two stimulation conditions. To evoke neurogenic contractions, a stimulus of 100V intensity, 1 msec duration, 5Hz frequency, and 15 pulses was used. The response to this was completely abolished by treatment with 3 × 10^{-7}M TTX. A single pulse of 50V intensity, 1 sec duration was applied to stimulate the smooth muscle directly. A resting interval of over six minutes was required between stimulations to ensure that each contraction achieved the same amplitude.

Figure 2 shows the effect of adenosine on contractions generated by nerve stimulation and by direct muscle stimulation in the same specimen. When nerve stimulation and direct muscle stimulation were applied alternately every 6 to 12 minutes, contractions of approximately the same amplitude occurred with both conditions. Twenty minutes after the first stimulation, 10^{-6}M adenosine was superfused for 16 minutes. Each column represents the mean values of relative amplitude of contraction as normalized to the first contraction of each condition in the absence of adenosine. 10^{-6}M adenosine inhibited the contractions produced by nerve stimulation, without affecting those produced by direct muscle stimulation (contractions induced by nerve stimulation: 0.51 ± 0.09, n = 6, p < 0.01; and 0.53 ± 0.17, n = 6, p < 0.01, respectively). After washing of adenosine, contractions generated by nerve stimulation recovered.

**Effect of Adenosine on Neurogenic Contractions of CCA**

Transmural nerve stimulation (100V intensity, 1 msec duration, 5Hz; 10Hz and 20Hz, in frequency, 3 sec train duration) evoked frequency-dependent contractions. As shown in figure 3A, 10^{-6}M and 10^{-5}M adenosine inhibited neurogenic contractions at each frequency. When the contraction evoked by 20Hz frequency in the absence of adenosine was normalized as 1.0, the contractions at 20Hz in the presence of 10^{-6}M and 10^{-5}M adenosine were 0.89 ± 0.11, n = 6, p < 0.01; and 0.53 ± 0.17, n = 6, p < 0.01, respectively. The effect of adenosine on neurogenic contractions was more prominent in the low frequency range. When the contraction in the absence of adenosine in each frequency was registered as 1.0, the contractions at 5Hz and 20Hz in the presence of 10^{-6}M adenosine were 0.25 ± 0.17, n = 6; and 0.53 ± 0.17, n = 6, p < 0.01, respectively. Figure 3B shows an actual record of nerve stimulated contractions, demonstrating the inhibitory effect of 10^{-4}M and 10^{-5}M adenosine.
We examined the antagonistic action of theophylline on endogenous and exogenous adenosine. Theophylline itself in concentrations over $10^{-4}$ M, had inhibitory action on high [K]o induced (0.94 ± 0.03, n = 3) and NE induced (0.35 ± 0.05, n = 3) contractions. $10^{-5}$ M theophylline did not increase the amplitude of neurogenic contractions evoked by a 10H stimulus of the type described above (1.01 ± 0.04, n = 8). However, as shown in figure 4, $10^{-5}$ M theophylline had a slight antagonistic action on the inhibitory effect of adenosine, more so as low concentrations of adenosine ($10^{-6}$ M adenosine: 0.67 ± 0.08, n = 6; $10^{-4}$ M adenosine pretreated with $10^{-5}$ M theophylline: 0.84 ± 0.09, n = 9, p < 0.01) (fig. 4).

To investigate the influence of dipyridamole on adenosine's inhibitory effect on neurogenic contractions, we applied dipyridamole in the absence, and presence of adenosine, and evoked neurogenic contractions with a 10H stimulus as described above. Dipyridamole (in concentrations over $2 \times 10^{-5}$ M) itself had a vasodilating action on high [K]o induced (0.81 ± 0.02, n = 3) and NE-induced (0.31 ± 0.02, n = 3) contractions. At a concentration of $2 \times 10^{-4}$ M, dipyridamole did not affect either high [K]o induced, NE-induced, or neurogenic contractions. However, it augmented the inhibitory action of adenosine on neurogenic contraction as shown in figure 5A and B. By itself, $10^{-3}$ M adenosine had no inhibitory action on neurogenic contractions, but in the presence of $2 \times 10^{-5}$ M dipyridamole, the amplitude of neurogenic contractions was decreased ($10^{-7}$ M adenosine: 0.99 ± 0.03, n = 6; $10^{-5}$ M adenosine with $2 \times 10^{-5}$ M dipyridamole: 0.78 ± 0.06, n = 9, p < 0.01).

**Discussion**

Adenosine has a direct vasodilating action on canine vascular smooth muscle, and the potency of its action varies by region. In the intracranial vessels, there is a slight difference in the potency of the vasodilating action of adenosine between basilar and middle cerebral arteries. In human basilar artery, even $10^{-3}$ M adenosine does not produce a vasodilating effect. In this experiment, the concentration of adenosine required to relax the high [K]o-induced and NE-induced contractions of the CCA was higher than that required for peripheral arteries. Herlihy, et al mentioned that adenosine's efficacy decreased with increasing concentrations of the stimulating agent. However, the concentration of high [K]o solution in this experiment was 39.2 mM, which only produced about half of the maximum contraction evoked by full depolarization of the muscle cells. Therefore, it is most likely that the observations reported herein can be explained by regional differences in vascular reactivity.

Theophylline is a commonly used postsynaptic an-
tagonist of adenosine. Muramatsu et al. reported theophylline was effective for the relaxing response only induced by low concentrations of adenosine on canine middle cerebral artery, and concluded that theophylline was not a true competitive blocker of adenosine. In this experiment, concentrations of theophylline over $3 \times 10^{-5}$M alone had a nonspecific relaxing action on smooth muscle cells in the CCA, and the relaxing action of adenosine on contractions evoked by vasoactive substances was little affected by $10^{-3}$M theophylline.

To examine the effect of adenosine on contraction evoked by electrical stimulation, we should confirm that the contractions evoked by electrical stimulation were neurogenic, and eliminate the effect of adenosine on the postsynaptic membrane of smooth muscle cells. Since the innervation of peripheral vascular smooth muscle is adrenergic, the effect of adenosine on neurogenic contraction is usually compared to the effect of adenosine on contractions evoked by exogenously applied norepinephrine in peripheral vascular beds. However, we determined that the neurogenic contraction of the CCA had different characteristics from that of peripheral arteries, ie, the contraction of the CCA was abolished, as in peripheral arteries, by blockade of peripheral sympathetic transmission with guanethidine or 6-OHDA, but was not inhibited by the $\alpha$-blocking agents prazosin or yohimbine (Fujisawa, S, unpublished data). We proposed that the neuromuscular transmission of canine CCA was essentially sympathetic, but there was some possibility that an unknown transmitter or different type of adrenoceptor was participating in neuromuscular transmission (Fujisawa, S, unpublished data). Therefore, to eliminate the effect of adenosine on the postsynaptic membrane of smooth muscle, we examined the effect of adenosine on the postsynaptic membrane of smooth muscle, we examined the effect of adenosine on contractions induced by high [K]o, direct muscle stimulation, and NE.

These experiments demonstrated the inhibitory effect of adenosine on the response to electrical nerve stimulation at concentrations that were too low to directly affect the response of the vascular smooth muscle of CCA to high [K]o solution, exogenous NE, and direct muscle stimulation. This preferential inhibition of contractile response evoked by transmural nerve stimulation implies that the adenosine is acting at a presynaptic receptor.

In peripheral arteries, theophylline had an action antagonistic to adenosine in neuromuscular transmission. In CCA, $10^{-3}$M theophylline had an action antagonistic to the presynaptic inhibitory effect of adenosine, but the extent of this antagonistic action was much less than in peripheral artery. Since concentrations of theophylline over $3 \times 10^{-3}$M act directly on smooth muscle, we could not use such concentrations to evaluate the presynaptic antagonism between adenosine and theophylline. This suggests that there is a presynaptic adenosine receptor in the CCA, but that it has properties slightly different from those of receptors in peripheral arteries.

The experiment with dipyridamole was designed to see if the effects of endogenous adenosine could be enhanced. Dipyridamole, which inhibits adenosine uptake and hence its inactivation, inhibited not only neurogenic contractions but also high [K]o solution induced and NE-induced contractions in this tissue. This means that dipyridamole has non-specific vasodilating effect, which was not due to the solvent effect of PEG. Accordingly, we could not conclude that the inhibitory action of dipyridamole on neurogenic contraction was only due to the enhanced action of endogenous adenosine. However, the finding that $2 \times 10^{-5}$M dipyridamole potentiated the inhibitory action of adenosine without having any effect on the postsynaptic membrane of smooth muscle cells suggests that dipyridamole was acting via adenosine receptors as observed in peripheral vascular beds or coronary arteries. It also suggested that adenosine acts at an extracellular site, and that the CCA possesses an adenosine uptake process as reported in several rabbit vascular tissues and rat vas deferens.

Katsuragi and Su reported that theophylline augmented purine release from vascular adrenergic nerves and suggested the presence of presynaptic autoinhibition of purine compounds in rabbit pulmonary artery. In this experiment, at concentrations which did not affect postsynaptic smooth muscle cells, theophylline had no augmenting effect on neurogenic contraction of the CCA. Our findings concerning the effect of both dipyridamole and theophylline on neurogenic contraction of the CCA in the absence of adenosine exogenous, do not support the presence of autoinhibition of adenosine in neuromuscular transmission. Nevertheless, since we have demonstrated that adenosine receptors can modify transmitter release, and it is known that released ATP is rapidly hydrolysed to adenosine, it seems that endogenous adenosine could modify transmitter release under certain conditions.

In conclusion, there is a presynaptic adenosine receptor in the nerve terminal of canine CCA which inhibits the release of neurotransmitter, but endogenously released adenosine from nerve terminal does not appear to play a major role in this tissue. Adenosine has a vasodilating effect on canine CCA which is mainly due to its inhibitory effect on the neuromuscular transmission, rather than to its direct relaxing effects on smooth muscle.

References


Presynaptic inhibitory action of adenosine on neuromuscular transmission in the canine cavernous carotid artery.
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