Bicarbonate-Dependent Action of Bay K 8644 in the Smooth Muscle of the Rat Vas Deferens

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Abstract—Effects of Bay K 8644, partial depolarization with high potassium, and nifedipine on the dose-response curves of the rat vas deferens to norepinephrine, methacholine and KCl were investigated in HEPES-buffered physiological salt solution (PSS) with or without 20 mM sodium bicarbonate. In the bicarbonate-containing PSS, Bay K 8644 at $10^{-6}$ M enhanced the maximal contractions in response to norepinephrine, methacholine and KCl by 31.4, 103.3 and 40.1%, respectively. In the bicarbonate-free PSS, where the maximal contractions induced by norepinephrine, methacholine and KCl were 77.5, 75.0 and 68.2% of those in the bicarbonate-containing PSS, respectively, Bay K 8644 did not enhance the maximal contractile response to any of the agonists, although the contractions induced by low concentrations of KCl were increased by Bay K 8644. Increasing the potassium concentration in the PSS from 6 to 20 mM enhanced the maximal contractions in response to norepinephrine and methacholine in the bicarbonate-containing PSS, whereas in the bicarbonate-free PSS, the treatment decreased the contractions. In the two PSSs, nifedipine similarly inhibited the contraction in response to either $10^{-6}$ M norepinephrine or 68 mM KCl. These results suggest that bicarbonate ion modulates the function of the voltage-dependent calcium channel in the smooth muscle of the rat vas deferens.

We have recently reported (1) that in the rat vas deferens, cocaine increased the sensitivity to norepinephrine and enhanced the contractions in response to norepinephrine, methacholine and KCl in the HEPES-buffered physiological salt solution (PSS) containing sodium bicarbonate. On the other hand, in the bicarbonate-free PSS, cocaine did not enhance the contractile response to either agonist, although cocaine still increased the sensitivity to norepinephrine to almost the same extent as that in the bicarbonate-containing PSS. We have suggested from these results that bicarbonate is crucial for the postjunctional, but not for the presynaptic action of cocaine in the rat vas deferens.

It was noted in our previous study (1) that the magnitudes of maximal contractions for the three agonists, in particular, KCl, were reduced in the bicarbonate-free solution, indicating that the calcium influx through the voltage-dependent calcium channel was diminished in the bicarbonate-deprived PSS. In the present study, the effects of Bay K 8644, nifedipine and partial depolarization of the membrane potential with high potassium on the agonist-induced contractions were examined in the bicarbonate-containing and bicarbonate-free PSS to investigate the role of bicarbonate ion in the functions of the voltage-dependent calcium channel in the rat vas deferens.

Materials and Methods
A pair of vasa deferentia from male Wistar rats (250–350 g) were taken and placed in a petri dish containing HEPES-buffered PSS with or without bicarbonate ion. The composition of the bicarbonate-containing PSS was as follows: 120 mM NaCl, 6 mM KCl, 2
mM CaCl₂, 1 mM MgCl₂, 1 mM NaH₂PO₄, 20 mM NaHCO₃, 5 mM HEPES and 5.5 mM glucose; the pH was adjusted to 7.3 at 36°C by 1 N HCl. Bicarbonate-free PSS contained 140 mM NaCl instead of 120 mM NaCl, and the other components were the same as those in the bicarbonate-containing PSS; the pH was adjusted to 7.3 by 1 N NaOH. High KCl solutions were made by replacing NaCl with equimolar KCl.

After being cleaned of adhering fat and blood vessels, the epididymal portion of approximately 10 mm was mounted in an organ chamber of 10-ml capacity with the resting tension of 0.5 g and allowed to equilibrate for 60 min. In the experiments to determine the contractions in response to KCl, the preparations were treated with 10⁻⁶ M dipenamine for 10 min during the equilibration period. The bathing solution in the organ chamber was maintained at 36°C and continuously exchanged with the fresh solution at the rate of 3 ml/min. The bathing solution was bubbled with pure oxygen.

The dose-response curves for the agonists were obtained in a non-cumulative way. Increasing concentrations of the agonists were applied with an interval of 15 min between the applications. The peak amplitudes of the contractions were used to construct the dose-response curve. When the effects of Bay K 8644 or the increase of potassium concentration in the PSS from 6 to 20 mM on the dose-response curve were examined, control responses without the treatments were first determined, the preparations were allowed to rest for 30 min, and then the responses in the presence of 10⁻⁶ M Bay K 8644 or in the 20 mM potassium-containing PSS were determined. In the experiments to examine the effects of Bay K 8644 and nifedipine on the agonist-induced contractions, the contractions were evoked with an interval of 20 min, and Bay K 8644 or nifedipine was applied to the organ chamber 10 min prior to each contraction.

Obtained results were statistically evaluated by Student's non-paired t-test, and the P value of 0.05 was adopted to assess the significant difference.

Bay K 8644 (Bayer A.G.) and nifedipine (Yodogawa Seiyaku) were dissolved in ethanol, and the final concentration of ethanol in the organ chamber was less than 0.1%. Experiments using Bay K 8644 and nifedipine were done under a dim light. The other drugs used were the same as those in the previous study (2).

![Dose-response curves to norepinephrine (NE), methacholine (MCh) and KCl (K) in the HEPES-buffered physiological solution containing sodium bicarbonate (20 mM HCO₃) and bicarbonate-free solution (0 mM HCO₃). The number of preparations tested is 12 for all experiments. Vertical bars represent standard errors of the mean. Asterisks indicate that there is a significant difference in the values between the two solutions.](image)
Results
Effects of bicarbonate on the agonist-induced contractions: Figure 1 shows the dose-response curves to norepinephrine, methacholine and KCl in the 20 mM sodium bicarbonate-containing and bicarbonate-free PSS. In the bicarbonate-free PSS, the contractions in response to high concentrations of norepinephrine, methacholine and KCl were significantly smaller than those in the bicarbonate-containing one, while the contractions induced by low concentrations of the agonists were similar in the two PSSs. Compared with the drug agonists, the KCl-induced contractions were reduced in the entire range of the concentration and the reduction in the maximal response was greater than those for norepinephrine and methacholine. The maximal contractile response to KCl in the bicarbonate-free PSS was 68.2% of that in the bicarbonate-containing PSS, being smaller than 77.5 and 75.0%, the corresponding values for norepinephrine and methacholine.

Effects of bicarbonate on the action of Bay K 8644: Effects of 10^-6 M Bay K 8644 on the dose-response curves to the three agonists in the bicarbonate-containing and bicarbonate-free PSS are shown in Fig. 2. In the bicarbonate-containing PSS (upper panel of Fig. 2), Bay K 8644 enhanced the maximal contractions in response to norepinephrine by 31.4%, and it increased the sensitivity by 13.5-fold as
Fig. 3. Effects of various concentrations of Bay K 8644 on the contractions induced by 15 mM KCl in the presence and absence of 20 mM sodium bicarbonate (20 mM HCO$_3^-$ and 0 mM HCO$_3^-$). Shown at "C" are the values obtained without Bay K 8644 treatment. Vertical bars represent standard errors of the mean (n=5).

Fig. 4. Effects of increasing the potassium concentration in the bathing solution from 6 mM (control) to 20 mM (20 mM K) on the dose-response curves to norepinephrine (NE) and methacholine (MCh) in the presence of 20 mM sodium bicarbonate (upper panel) and absence of bicarbonate (lower panel). The number of preparations is 6 for all experiments. Vertical bars represent standard errors of the mean. Asterisks indicate that there is a significant difference between the control and 20 mM potassium-treated preparations.
represented by the shift of ED50. Bay K 8644 also enhanced the maximal contractions in response to methacholine by 103.3%, but Bay K 8644 did not affect the sensitivity to methacholine. Bay K 8644 markedly enhanced the KCl-induced contractions in the bicarbonate-containing PSS. After the treatment with Bay K 8644, 20 mM KCl, which was the marginal concentration to induce contractions in the control condition, produced contractions of almost the maximal amplitude. The maximal contractile response to KCl was increased by Bay K 8644 by 40.1% in the bicarbonate-containing PSS.

In the bicarbonate-free PSS (lower panel of Fig. 2), Bay K 8644 increased the contractions in response to low concentrations of norepinephrine and methacholine, but the increment was not significant. The contractions in response to high concentrations of the agonists were rather decreased by Bay K 8644. Bay K 8644 slightly enhanced the contractions in response to low concentrations of KCl in the bicarbonate-free PSS, whereas the maximal contractile response to KCl was reduced by Bay K 8644.

The dose-effect relationship for the enhancing action of Bay K 8644 was compared in the two PSSs by assessing the enhancement by various concentrations of Bay K 8644 toward the contractions induced by 15 mM KCl. As shown in Fig. 3, Bay K 8644 at more than $10^{-9}$ M dose-dependently increased the contractions in the two PSSs. However, the extent of the enhancement was very much greater in the bicarbonate-containing PSS than in the bicarbonate-free PSS.

Effects of 20 mM potassium on the contractions in the two PSSs: Effects of increasing the potassium concentration in the PSS from 6 to 20 mM on the dose-response curves to norepinephrine and methacholine are shown in Fig. 4. In the bicarbonate-containing PSS (Fig. 4, upper panel), 20 mM potassium increased the contractions in response to every concentration of the two drugs, although the increment was not necessarily significant. In the bicarbonate-free PSS (lower panel), the contractions in response to high concentrations of norepinephrine and methacholine were significantly depressed in the presence of 20 mM potassium, while the contractions induced by the low concentrations of agonists were not significantly affected by the elevated potassium concentration.

Effects of nifedipine in the two PSSs: Dose-inhibition curves for nifedipine on the contractions induced by $10^{-5}$ M norepinephrine and 68 mM KCl were determined in the bicarbonate-containing and bicarbonate-free PSS (Fig. 5). As shown in the upper panel of Fig. 5, the dose-inhibition curves of nifedipine for the norepinephrine-induced contractions were almost identical in the two PSSs. The negative logarithm of the IC50 was 7.51 and 7.61 for the bicarbonate-containing and bicarbonate-free PSS, respectively.

The high KCl-induced contractions were
more sensitive to nifedipine than norepinephrine-induced contraction by approximately 10-fold in the two PSSs (Fig. 5, lower panel). The negative logarithm of the IC50 was 8.45 and 8.29 for the bicarbonate-containing and bicarbonate-free PSS, respectively.

**Discussion**

It has been reported that Bay K 8644 enhanced the contractions induced by KCl and drug agonists acting on the receptors in several smooth muscles such as the rabbit mesenteric artery (3), rat fundus (4), rat saphenous vein (5) and rabbit aorta (6–8). The bathing solutions used in these studies contained 10–25 mM bicarbonate ion, but the effects of omitting bicarbonate were not described therein. In the rabbit aorta, Bay K 8644 was reported to potentiate the contractions in response to KCl or BaCl2 in the bicarbonate-free solution (9, 10). Hence bicarbonate may not be essential for the action of Bay K 8644 in this smooth muscle. However, it can not be concluded that bicarbonate is also not necessary for the action of Bay K 8644 in other smooth muscles. The present study showed that the enhancing action of Bay K 8644 was strongly dependent on the presence of bicarbonate in the rat vas deferens. Furthermore, the enhancement of the drug-induced contractions by partial depolarization of the membrane potential with high potassium was also dependent on the presence of bicarbonate. In contrast, the inhibitory action of nifedipine was independent of bicarbonate. These results suggest that the stimulation process of the voltage-dependent calcium channel is modulated by bicarbonate ion in the rat vas deferens.

Although it cannot be ruled out that bicarbonate directly affects the voltage-dependent calcium channel in the rat vas deferens, there is too little information to evaluate this possibility. Bicarbonate may affect the calcium channel indirectly through modifying the status surrounding the calcium channel, since bicarbonate is known to be involved in several cellular functions such as regulation of pH (11) and the ion transport process of chloride in the vas deferens (12). A plausible indirect action of bicarbonate involves the effect of pH: A change in pH has been suggested to produce the conformational change in the L-type calcium channel in guinea pig ventricular cells (13).

Since Bay K 8644 and nifedipine, respectively, enhanced and inhibited the contractions in response to norepinephrine in the rat vas deferens, it seems reasonable to presume that the contractions induced by norepinephrine are entirely or partially due to the influx of calcium through the dihydropyridine calcium modulator-sensitive voltage-dependent calcium channel. From the result that nifedipine greatly inhibited the norepinephrine-induced contractions in the rat vas deferens. Han et al. (14) suggested that norepinephrine-produced contractions were largely due to the influx of calcium through the nifedipine-sensitive calcium channel, although the mechanism of activating the calcium channel by norepinephrine was not clarified in their study. On the other hand, Khoyi et al. (15) proposed that norepinephrine brought about the contractions primarily by releasing calcium from the intracellular storage sites via the formation of inositol 1,4,5-trisphosphate. The present study confirmed the results reported by Han et al. (14); thus we suppose that norepinephrine increased the influx of extracellular calcium through the nifedipine-sensitive calcium channel in the rat vas deferens. The increase in calcium-influx through the dihydropyridine-sensitive calcium channel may also be involved in the methacholine-induced contraction in the rat vas deferens.

The norepinephrine-induced contractions were approximately 10 times less sensitive to nifedipine than the KCl-induced contractions. This fact appears to indicate that norepinephrine and KCl opened different kinds of nifedipine-sensitive calcium channels in the rat deferens. However, only a single binding site for [3H]-nimodipine was revealed with Scatchard plot analysis in the sarcolemmal membranes of the rat vas deferens (16), suggesting that norepinephrine and KCl activated the same nifedipine-sensitive calcium channel.

The difference in the sensitivity to nifedipine between norepinephrine and KCl may be related to the two agonists having different mechanism for opening the calcium-channel.
As described before, the mechanism by which norepinephrine activates the calcium channel is still obscure in the rat vas deferens. However, it seems possible that norepinephrine activates the nifedipine-sensitive calcium channel not only by depolarizing the membrane but also by a depolarization-independent mechanism. On the other hand, KCl may open the calcium channel mainly by depolarizing the membrane. Recently, using the patch clamp technique in the isolated smooth muscle cell of the rat vas deferens, Nakazawa et al. (17) have reported that nicardipine blocked almost fully the Ba current when the current was induced from the holding potential of −30 mV, whereas it only slightly blocked the current induced from the holding potential of −80 mV. Their results indicate that the sensitivity of the calcium channel to dihydropyridine antagonists may vary depending on the membrane potential. If this is the case, the different sensitivity between norepinephrine and KCl to nifedipine may be accounted for by the differential effects of the two agonists on the membrane potential. The KCl-induced contractions would be more sensitive to nifedipine due to its rapid depolarizing action on the membrane potential.

In conclusion, the present study demonstrated that bicarbonate may affect the stimulation process of the dihydropyridine-sensitive calcium channel by Bay K 8644 and the depolarization in the rat vas deferens. The mechanism for this action of bicarbonate remains to be studied.

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