ARTIFICIAL BUFFERS DO NOT INHIBIT CONTRACTILE RESPONSES IN THE SMOOTH MUSCLE OF RAT PORTAL VEIN AND GUINEA PIG TAENIA COLI

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Abstract—Effects of substitution for NaHCO₃ (and 5% CO₂) in the physiological solution with equimolar N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) or morpholinopropane sulfonic acid (MOPS) (and 100% O₂) at various pH levels on the contractility of smooth muscle were examined. At pH 7.4, spontaneous contraction in rat portal vein was not inhibited by the artificial buffer solutions, as compared to findings in the case of bicarbonate buffer solution. Norepinephrine-dose response curve in the case of rat portal vein and the histamine-dose response curve in cases of guinea pig taenia coli remained unchanged in the artificial buffer solutions. At pH 7.2 and 7.0, the spontaneous contraction in portal vein was reversibly inhibited either in artificial or in bicarbonate buffer solutions. The norepinephrine- and histamine-dose response curves shifted downwards and/or to the right in these low pH solutions. Thus, HEPES and MOPS had no inhibitory effect on the smooth muscle contractility. Since low pH strongly inhibited the contractility, attention should be directed to the temperature-dependent decreases in pKa of the artificial buffers (i.e., if pH of the solution is adjusted at room temperature and then warmed, pH decreases).

Artificial buffer substances such as tris (hydroxymethyl) aminomethane (tris) and zwitterionic buffers have been widely used in various biological experiments when a bicarbonate buffer was not appropriate. These artificial buffers have also long been used in the studies of excitation-contraction coupling in smooth muscle. Recently, Altura and collaborators reported that the substitution for bicarbonate buffer system with tris or zwitterionic buffer systems markedly attenuated spontaneous mechanical activity in rat portal vein and also inhibited agonist-induced contractions in rat portal vein and aorta (1-3). Since the reduction in the concentration of HCO₃⁻ under CO₂ aeration (pH maintained at 7.4 by adding NaOH) had little effect on the smooth muscle contraction (3), they concluded that the artificial buffer substances have a strong inhibitory effect on the smooth muscle contractility (1-3). However, their experimental plan to reduce external NaHCO₃ concentration without changing pH is not appropriate since the addition of NaOH in the presence of CO₂ aeration results in a formation of NaHCO₃. Further, in previous studies (4-7), we found no inhibitory effect of tris on the smooth muscle contractions induced under different circumstances. In the present report, we examined the effects of N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid...
(HEPES) and morpholinopropane sulfonic acid (MOPS) on the contractility of rat portal vein and guinea pig taenia coli.

MATERIALS AND METHODS

Longitudinal segments of portal vein (approximately 5 mm long) were dissected from decapitated Wistar rats weighing 250–300 g. Pieces of taenia coli (20–25 mm long) were also isolated from male guinea pigs weighing 250–300 g. The muscle strips were isometrically mounted under a resting tension of 500 mg for portal vein and 200 mg for taenia.

The bicarbonate buffer solution contained (mM) NaCl 136.8, KCl 5.4, CaCl₂ 2.5, MgCl₂ 1.0, glucose 5.5 and NaHCO₃ 25.0 and was aerated with 95% O₂ and 5% CO₂ mixture at 37°C and pH 7.4. In some experiments, the concentration of NaHCO₃ was reduced to 12.0 mM or to 6.0 mM in order to decrease the pH of the solution to 7.2 or 7.0, respectively. Artificial buffer solutions were prepared by replacing the 25 mM NaHCO₃ in the above solution with equimolar HEPES or MOPS and neutralized to pH 7.4, 7.2 or 7.0 with NaOH and were aerated with 100% O₂. Since pKa values for HEPES and MOPS decrease with the increase in temperature (−0.014/°C for HEPES and −0.006/°C for MOPS), as is the case with tris (−0.031/°C), pH of these solutions was carefully adjusted at the experimental temperature (37°C).

Cumulative dose-response curves to nor-adrenaline (NE) and to histamine were obtained initially in bicarbonate buffer solution with pH 7.4 and subsequently in the modified solutions after a 60 min incubation in the latter solution. Although we confirmed in preliminary experiments that the dose-response curves were reproducible in the bicarbonate buffer solution with pH 7.4, only one of the modified solutions was tested only once with each muscle preparation to avoid possible degeneration in the contractility in the modified solutions. The Student’s t-test was used to obtain significant differences.

The chemicals used were HEPES (Wako Pure Chemicals Industries, Ltd., Tokyo), MOPS (Sigma Chemicals Co., St. Louis, M.O.), NE (bitartrate, Wako) and histamine (dihydrochloride, Wako).

RESULTS

Effects of buffer substances and pH on spontaneous contraction in portal vein: In bicarbonate buffer solution with pH 7.4, the portal vein showed spontaneous rhythmic contractions with an average frequency of 2.7±0.2/min and an average tension of 490±30 mg (n=20). When incubating the portal vein with HEPES buffer solution with pH 7.4, the frequency of the spontaneous contractions transiently increased. However, 5–10 min after changing the solution, both frequency and tension returned to the levels in the bicarbonate buffer solution (2.9±0.5/min and 515±45 mg, n=5). Typical results of the experiments are shown in Fig. 1 (upper trace). Similar results were obtained

Fig. 1. Effects of HEPES buffer solution with pH 7.4, 7.2 or 7.0 on spontaneous contractions in isolated rat portal vein. The portal vein was initially equilibrated in bicarbonate buffer solution with pH 7.4. Subsequently, the solution was changed with HEPES buffer solution with pH 7.4, 7.2 or 7.0.
with MOPS buffer solution with pH 7.4 (2.2±0.4/min and 506±61 mg, n=5). These results suggest that neither absence of bicarbonate ion nor addition of 25 mM HEPES or MOPS has any inhibitory effect on the spontaneous activity of portal vein. When decreasing the pH of the HEPES buffer solution to 7.2, the average spontaneous contractile tension decreased to 354±27 mg (n=5) and the shape of the contractions sometimes changed as shown in Fig. 1 (middle trace). Further decrease in pH to 7.0 more strongly inhibited the spontaneous contraction (Fig. 1, lower trace). Both tension and frequency of the spontaneous contractions returned to the control level when the pH of the solution was increased to 7.4. Similar results were obtained with bicarbonate buffer solution and MOPS buffer solution with low pH. Thus, low pH markedly attenuated the spontaneous contractions, irrespective of the buffer substances examined.

In taenia, spontaneous contractions of irregular shape and size were observed in bicarbonate buffer solution with pH 7.4. The spontaneous activity did not change in HEPES or MOPS buffer solution with pH 7.4 although the activity was greatly attenuated in the low pH solutions.

Effects of buffer substances and pH on NE-induced contraction in portal vein and histamine-induced contraction in taenia coli: As shown in Fig. 2, NE-dose response curves in case of the portal vein were not affected by the change in buffer substances at pH 7.4. Decreasing the external pH to 7.2 and 7.0, the dose response curves shifted to the right and/or to the downwards, irrespective of the buffer substances used. As shown in Fig. 3, similar results were obtained in histamine-dose response curves in case of the taenia coli.

DISCUSSION
Since Altura and collaborators reported
the strong inhibitory effects of tris on the contractile response as well as on the uptake of $^{45}\text{Ca}$ in smooth muscle of rat portal vein and aorta (1–3, 8), different investigators questioned their conclusion. Johansson et al. (9) did not find any inhibitory effect of tris on the spontaneous contractility of rat portal vein and suggested that the pH of the tris buffer solution used in the Altura’s laboratory might be lower than that of the bicarbonate buffer solution. The temperature-dependent change in pKa of tris is $-0.031/\degree\text{C}$ and if the pH of the tris buffer solution was adjusted to 7.4 at room temperature (20°C) and then warmed to 37°C, the pH would decrease to 6.87. Further, no inhibitory effect of tris was found in rat tail artery (10), rabbit main pulmonary artery (11), rabbit aorta (6) and in rat portal vein and aorta (7). On the effect of tris on $^{45}\text{Ca}$ movement, Turlapaty et al. (3, 8) compared the bicarbonate buffer solution containing phosphate ion and the tris buffer solution without phosphate ion and reported $^{45}\text{Ca}$ retention by smooth muscle was less in the tris buffer solution. Karaki and Weiss (6) examined the effects of phosphate ion on $^{45}\text{Ca}$ retention and concluded that phosphate increases $^{45}\text{Ca}$ retention by smooth muscle either in bicarbonate buffer or in tris buffer solution. They (6) suggested that the observation by Turlapaty et al. (3, 8) is not attributable to the inhibitory effect of tris but rather to the effect of phosphate ion.

The results obtained in the present experiments indicate that not only tris but also HEPES and MOPS have no inhibitory effect on the contractility of two different types of smooth muscle and that the absence of bicarbonate ion does not attenuate the contractility. It was also reported that histidine buffer has no inhibitory effect on the contractions in rabbit taenia (12). The only difference between bicarbonate buffer and artificial buffers reported so far is that the intracellular pH of smooth muscle was lower in bicarbonate buffer solution than that in tris buffer solution although pH of both solutions were identical (5). Such a

![Graphs of Bicarbonate, HEPES, and MOPS Buffer Solutions](image-url)
difference in intracellular buffering might affect smooth muscle function (5) although the contractility was not affected. The present results also confirmed earlier reports (12–15) that decrease in pH of the solution strongly attenuates smooth muscle contractility. The inhibitory effect of low pH is explained by a H⁺-Ca²⁺ competition which inhibits Ca²⁺ influx (15). Since the pKa value of most of the artificial buffer substances decrease with the increase in temperature, care should be taken to adjust the pH of the artificial buffer solutions at the experimental temperature. The temperature-dependent change in pKa of MOPS is small (−0.006/°C). We have no other explanation for the difference in conclusions obtained by Altura et al. (1) and our own group.

REFERENCES


