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Short communication

Hypocapnic constriction in rabbit basilar artery in vitro: triggering by serotonin and dependence on endothelin-1 and alkalosis

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Abstract

This study tested whether hypocapnic constriction of the rabbit basilar artery in vitro can be triggered by serotonin, and whether the resulting constriction is (1) due to the alkaline pH associated with hypocapnia, and (2) endothelin-1 mediated. Hypocapnic alkaline solution (25 mM $NaHCO_3$; pH 7.76; pCO_2 14.2) or isocapnic alkaline solution (50 mM $NaHCO_3$; pH 7.73; pCO_2 35.0) rarely altered basal tension. Serotonin (3 μM) challenge in hypocapnic or isocapnic alkaline solution resulted in near maximal tension. Washout of the serotonin did not decrease tension in 54% of the tissues, as plateau tension was maintained for 2–2.5 h. The plateau tension of washed tissues was relaxed by 1–3 μM PD145065 (Ac-D-Bhg-L-Leu-Asp-L-Ile-L-Ile-L-Trp), BQ610 (homopiperidinyl-CO-Leu-D-Trp(CHO)-D-Trp), and BQ788 (*N-cis*-2,6-dimethyl-piperidinocarbonyl-L-γ-MeLeu-D-Trp ($COOCH_3$)-Nle), endothelin ET_A/ET_B, endothelin ET_A, and endothelin ET_B receptor antagonists, respectively. In contrast, serotonin-induced tension in normal solution (25 mM $NaHCO_3$; pH 7.42; pCO_2 36.9) was maintained for only 40 min (mean). These results demonstrate that (1) constriction due to hypocapnia in vitro can be triggered by serotonin and is endothelin-1 mediated and (2) alkaline pH in the absence of decreased pCO_2 is sufficient to elicit the constriction triggered by serotonin.

Keywords: Endothelin-1; Basilar artery; 5-HT (5-hydroxytryptamine, serotonin); Hypocapnia; Isocapnia; Alkaline

1. Introduction

Hypocapnic constriction of cerebral vessels represents a major mechanism for the regulation of cerebral blood flow (Traystman, 1997). While the mechanism underlying the in vivo hypocapnic constriction is not entirely clear, we recently proposed that hypocapnic constriction in vitro was dependent on smooth muscle membrane depolarization and endothelin-1 release (Zuccarello et al., 2000a). This proposal was based on our recent demonstration that the development of hypocapnic constriction in rabbit basilar artery was dependent on initial challenge with KCl, and endothelin receptor antagonists reversed the constriction (Zuccarello et al., 2000b). Additionally, nitric oxide synthase inhibition, which can induce membrane depolarization and endothelin-1 release (Zuccarello et al., 1993; Kourembanas et al., 1993; Mitsutomi et al., 1999), triggered endothelin-1 dependent hypocapnic constriction in the basilar artery (Zuccarello et al., 2000a).

As it was recently reported that serotonin constriction of human pial arteries and rabbit middle cerebral artery was mediated via endothelin-1 release (Thorin et al., 1997, 1998) and, furthermore, serotonin depolarized the rabbit basilar artery (Clark and Garland, 1993), the present study tested whether serotonin triggers hypocapnic constriction in the rabbit basilar artery in vitro, and the dependence of the constriction on endothelin-1 release as well as alkaline pH.

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2. Materials and methods

2.1. Tissue preparation

New Zealand White male rabbits (2.8–3.2 kg) were anesthetized with ketamine HCl (30 mg/kg i.m.), xylazine (6 mg/kg i.m.), and pentobarbital Na^+ (35 mg/kg i.v.). Following exsanguination, the brain was removed and placed in ice cold Krebs Ringer bicarbonate solution containing (mM): 199 NaCl, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 25 $NaHCO_3$, 11 glucose, 0.026 EDTA, 2.5 $CaCl_2$. The basilar artery was then removed and cut into ring segments (2 mm) with care taken to preserve the endothelium intact. Each ring segment was placed in an organ bath containing 15 ml normocapnic (maximal bubbling with 94% O_2/6% CO_2; pH 7.42 ± 0.01, pCO_2 36.9 ± 0.3, pO_2 643.0 ± 6.7), hypocapnic (decreased bubbling with 94% O_2/6% CO_2; pH 7.76 ± 0.01, pCO_2 14.2 ± 0.2, pO_2 290.6 ± 6.5), or isocapnic alkaline Krebs Ringer bicarbonate solution (50 mM $NaHCO_3$ final using equimolar NaCl substitution; pH 7.73 ± 0.01; pCO_2 35.0 ± 0.4, pO_2 619.4 ± 4.8; means $\pm$ S.E.; $n = 21$, 28 and 12, respectively; from Zuccarello et al., 2000b). Although the pO_2 of the hypocapnic solution (291 mm Hg) was less than that of the normocapnic solution, this level of pO_2 is still relatively high and would not be expected to alter constriction. All solutions were prepared with 1 μM indomethacin.

Isometric tension was recorded by placing two tungsten wires (33 μm diameter) through the vessel lumen, with one wire attached to a microdrive and the other wire to a force displacement transducer. Vessel segments were placed at optimal resting tension (0.5-g force) and were allowed to equilibrate for 30–40 min prior to agent addition.

2.2. Protocol

Rings exposed to normal, hypocapnic alkaline, or isocapnic alkaline solution were challenged with 3 μM serotonin for 20 min. If significant tension did not develop, serotonin was washed out with the appropriate solution and immediately rechallenged with serotonin. The serotonin challenge/washout sequence was repeated for the number of times indicated. Serotonin was not washed out in some tissue rechallenged with serotonin, as indicated. Constricted tissues were then exposed to endothelin receptor antagonist, papaverine, elevated pCO_2, or acetylcholine.

2.3. Statistical methods

Statistical significance between two means was determined using Student's unpaired *t*-test and was accepted at the 0.05 level of probability. Values are expressed as means $\pm$ S.E. '*n*' represents the number of tissues.

2.4. Materials

Reagent sources were as follows: Peptides International for BQ610 (homopiperidinyl-CO-Leu-D-Trp(CHO)-D-Trp) and BQ788 (*N-cis*-2,6-dimethyl-piperidinocarbonyl-L-γ-MeLeu-D-Trp ($COOCH_3$)-Nle), Sigma for 5-hydroxytryptamine creatinine sulfate (serotonin), indomethacin, and papaverine HCl, and Parke-Davis Pharmaceutical for PD145065 (Ac-D-Bhg-L-Leu-Asp-L-Ile-L-Ile-L-Trp; gift).

3. Results

3.1. Serotonin and normal solution

In normal solution, 3 μM serotonin for 20 min induced tension in two of 10 tissues (see Section 2.2). Wash of the eight out of the 10 tissues that did not constrict in response to serotonin, followed by a second challenge with serotonin, resulted in the constriction of an additional four tissues. The magnitude of constriction in tissues challenged with serotonin was 3.7 ± 0.6 mN/mm length times two (mean $\pm$ S.E., $n = 6$; Fig. 1). Tension was maintained for 30 min in four tissues, and for 60 min in two tissues.

3.2. Serotonin and hypocapnic alkaline solution

In hypocapnic alkaline solution, four of 51 tissues developed tension (Zuccarello et al., 2000b). Serotonin (3 μM) for 20 min induced tension in 36 of 55 tissues exposed to hypocapnic alkaline solution. Extending the time of serotonin exposure from 20 to 40–50 min did not result in the development of tension in a greater number of tissues.

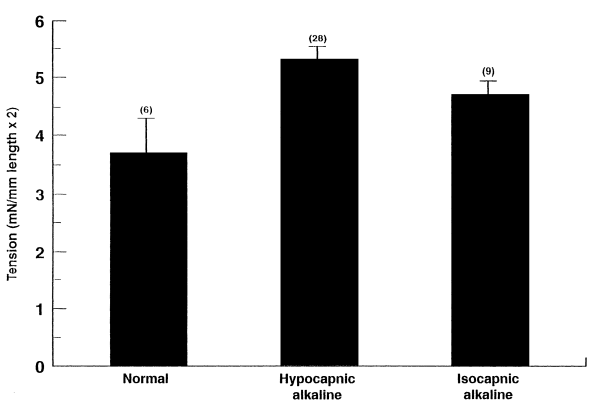

Fig. 1. Serotonin-induced constriction in normal, hypocapnic alkaline and isocapnic alkaline solution. Rabbit basilar artery rings were incubated in normal, hypocapnic alkaline and isocapnic alkaline solution, challenged with 3 μM serotonin, and then washed. Note that the duration of plateau tension elicited by serotonin in normal and hypocapnic/isocapnic alkaline solution was 40 and 120–150 min, respectively (text). Tension was calculated as mN/mm length times two. Values shown are mean $\pm$ S.E.; *n* is indicated in parenthesis.

In the 19 out of the 55 tissues that did not constrict following the initial serotonin challenge, wash with hypocapnic alkaline solution and rechallenge with 3 μM serotonin for 20 min resulted in the constriction of an additional 11 tissues. Third and fourth wash/serotonin challenges of the eight tissues that remained unconstricted by prior serotonin challenge, resulted in constriction of an additional four and two tissues, respectively. Thus, overall, 53 of 55 tissues constricted following one to four serotonin challenges in hypocapnic alkaline solution.

Washout of the serotonin with hypocapnic alkaline solution did not reduce tension in 28 of the 53 constricted tissues (Fig. 1), while the remaining tissues relaxed completely. Tension in washed tissues was maintained for 2–2.5 h. Tissues that relaxed upon serotonin washout with hypocapnic alkaline solution reconstricted following rechallenge with serotonin.

3.3. Serotonin and isocapnic alkaline solution

To investigate whether hypocapnia and/or alkalinity was necessary for serotonin to trigger the prolonged constriction observed in hypocapnic alkaline solution, tissues were exposed to isocapnic alkaline solution followed by serotonin. Isocapnic solution alone did not induce contraction (Zuccarello et al., 2000a). Serotonin (3 μM; 1–3 20 min challenges) increased tension in 15 of 15 tissues exposed to isocapnic alkaline solution. Eleven tissues developed tension following the initial serotonin challenge, while three and one tissues required second and third wash/serotonin challenges, respectively.

Washout of the serotonin with isocapnic alkaline solution did not reduce tension in nine of 15 constricted tissues (Fig. 1), while the remaining tissues relaxed completely. Tension in washed tissue was maintained for 2–2.5 h. Tissues that relaxed upon serotonin washout with isocapnic alkaline solution reconstricted following rechallenge with serotonin.

3.4. Endothelin receptor antagonists, increased pCO_2, and papaverine

PD145065, BQ610, and BQ788 (1 μM; endothelin ET_A/ET_B, endothelin ET_A, and endothelin ET_B receptor antagonists, respectively), relaxed the tension due to serotonin plus serotonin washout in hypocapnic alkaline solution by 77.8 ± 12.3% (6), 64.3 ± 15.3% (8), and 45.8 ± 17.3% (6), respectively (means ± S.E.; *n*). Similarly, 1 μM PD145065, BQ610, and BQ788 relaxed the tension due to serotonin plus serotonin washout in isocapnic alkaline solution by 54.0 ± 21.2% (3), 39.8 ± 3.5% (3), and 5.9% (2), respectively (means ± S.E.; *n*). Increasing the PD145065, BQ610, and BQ788 concentrations to 2, 3, and 4 μM, respectively, in tissues that were relaxed by 1 μM endothelin receptor antagonist by less than 60%, completely relaxed the remaining tension (Fig. 2A).

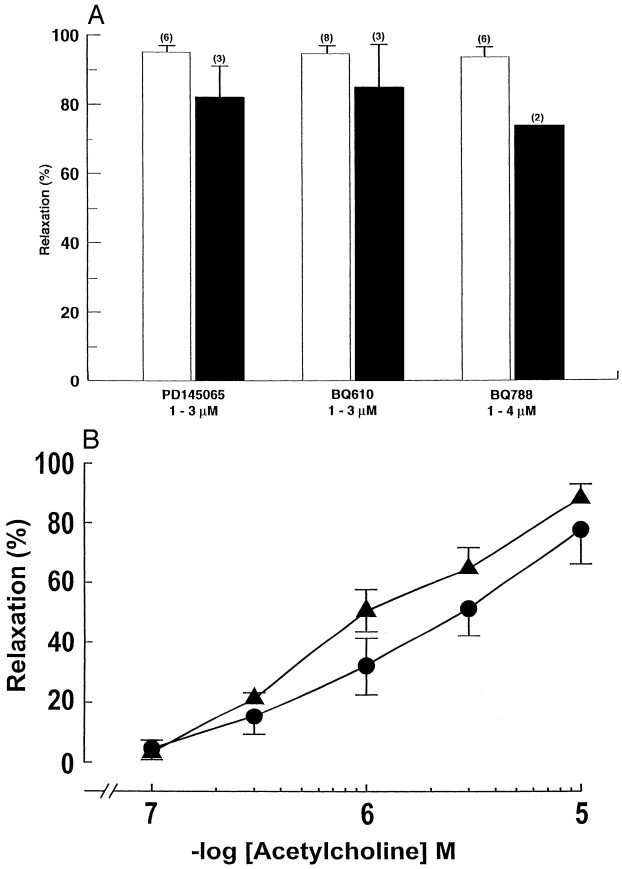

Fig. 2. Endothelin receptor antagonist and acetylcholine relaxation of the serotonin-triggered constriction in hypocapnic alkaline and isocapnic alkaline solution. Rabbit basilar artery rings incubated in (A) hypocapnic alkaline (open bar) and isocapnic alkaline solution (solid bar) were challenged with 3 μM serotonin followed by serotonin washout with hypocapnic alkaline or isocapnic alkaline solution, respectively. Constricted tissues were then challenged with 1–2 μM PD145065, 1–3 μM BQ610, or 1–4 μM BQ788. (B) hypocapnic alkaline solution were challenged with 3 μM serotonin followed by serotonin washout (circle), or in the presence of serotonin (triangle). Constricted tissues were then challenged with cumulative concentrations of acetylcholine. Results are expressed as percent relaxation of the tension. Values shown are mean ± S.E.; *n* is indicated in parenthesis.

Variable relaxant effects of the endothelin receptor antagonists were observed in serotonin constricted tissues exposed to hypocapnic alkaline or isocapnic alkaline solution and in which serotonin was not washed out. In hypocapnic alkaline solution, 3–4 μM PD145065, BQ610, and BQ788 failed to relax five of seven, two of five, and two of six tissues, respectively, within 1.5 h of endothelin receptor antagonist exposure. In isocapnic alkaline solution, 1–2 μM PD145065, 1 μM BQ610, and 2–4 μM BQ788 failed to relax two of two, one of two, and one of five tissues, respectively, within 1.5 h of endothelin receptor antagonist exposure. Attempts to determine the possible relaxant effects of endothelin receptor antagonists on the serotonin constriction in normal solution were inconclusive due to the relative lack of maintained constriction (see Section 3.1).

Return of hypocapnic alkaline solution to normal pCO_2 and pH by increased bubbling induced 96.5 ± 2.2% (mean ± S.E.; $n = 5$) relaxation of tension due to serotonin challenge in hypocapnic alkaline solution followed by serotonin washout with hypocapnic alkaline solution. In tissues exposed to hypocapnic alkaline solution and in the presence of serotonin, increased bubbling induced 97.0 ± 2.0% relaxation (mean ± S.E.; $n = 3$). Papaverine (30 μM) also relaxed by at least 96% the tension due to serotonin challenge in hypocapnic alkaline solution followed by serotonin washout with hypocapnic alkaline solution, and due to serotonin challenge in hypocapnic or isocapnic alkaline solution and in the presence of serotonin ($n = 1$ in each case). Acetylcholine (0.1–10 μM) concentration-dependently relaxed the tension due to serotonin challenge in hypocapnic alkaline solution followed by serotonin washout with hypocapnic alkaline solution, or in the presence of serotonin (Fig. 2B).

4. Discussion

The major finding of this study is that serotonin triggers endothelin-1 mediated hypocapnic constriction of the rabbit basilar artery in vitro. We propose that the mechanism whereby serotonin triggers constriction is via its ability to release endothelin-1 (Thorin et al., 1997, 1998) and induce depolarization (Clark and Garland, 1993). This proposal is consistent with our previous suggestion that hypocapnic constriction in vitro can be triggered by agents that release endothelin-1 and depolarize smooth muscle (Zuccarello et al., 2000a).

As serotonin triggered endothelin-1 dependent constriction in isocapnic alkaline solution, alkalinization, rather than decreased pCO_2, appears responsible for the prolonged constriction in hypocapnic alkaline solution. Additionally, the prolonged constriction appears entirely independent of decreased release of nitric oxide or possible other relaxant factors, as acetylcholine relaxed vessels exposed to hypocapnic alkaline solution and initially challenged with serotonin to the same magnitude as those initially challenged with KCl or N^G-monomethyl-L-arginine monoacetate (Zuccarello et al., 2000a,b).

The observations that selective endothelin ET_A and endothelin ET_B receptor antagonists relaxed the constriction that was maintained following serotonin washout suggest the following roles for endothelin-1 in the development and maintenance of the constriction. The constriction elicited in hypocapnic alkaline solution may be endothelin ET_A receptor mediated, as endothelin ET_A receptor antagonist, but not endothelin ET_B receptor antagonist, relaxed the constriction due to exogenous endothelin-1 (Zuccarello et al., 2000a). Thus, endothelin ET_B receptor antagonist relaxation of tissues following serotonin washout was not the result of blockade of endothelin ET_B receptor-mediated constriction. Rather, the relaxation may have been due to blockade of a positive feedback loop in which endothelin-1 induces further endothelin-1 release.

Consistent with the suggestion that the prolonged constriction triggered by serotonin in hypocapnic alkaline solution is the result of a positive feedback loop in which endothelin-1 induces further endothelin-1 release via endothelin ET_B receptor activation, are demonstrations that endothelin-1 can induce further endothelin-1 release and, moreover, the release is endothelin ET_B receptor mediated (Yokokawa et al., 1991; Saijonmaa et al., 1992; Fujitani et al., 1992; Iwasaki et al., 1995). Furthermore, we previously demonstrated that endothelin-1 challenge of basilar artery in hypocapnic alkaline solution triggered constriction that was maintained following repeated wash with hypocapnic alkaline solution and, moreover, the maintained constriction was endothelin-1 dependent as it was relaxed by BQ610 (Zuccarello et al., 2000b).

We currently do not have a clear explanation for the relaxation of a significant number of tissues following serotonin washout of tissues constricted in hypocapnic alkaline and isocapnic alkaline solution. These results differ from the constriction elicited by N^G-monomethyl-L-arginine monoacetate in tissues incubated in hypocapnic alkaline and isocapnic alkaline solution, in that tension was maintained in all tissues following N^G-monomethyl-L-arginine monoacetate washout with hypocapnic alkaline or isocapnic alkaline solution (Zuccarello et al., 2000a). It may be of interest to speculate that, in some tissues exposed to hypocapnic alkaline solution, the amount of endothelin-1 released by serotonin may be insufficient to trigger endothelin-1-induced endothelin-1 release and, thus, prolonged constriction following serotonin washout. It should also be considered that variability in the magnitude of depolarization due to serotonin (Clark and Garland, 1993) and alkalinization (Harder and Madden, 1985) may also contribute to the inability of some tissues to maintain constriction following serotonin washout in hypocapnic alkaline and isocapnic alkaline solution. Clearly, measurements of endothelin-1 release and membrane depolarization would greatly assist in determining their involvement in the constriction.

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