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Letter to the Editor

Endothelin-1 Mediates Hypocapnic Constriction of the Rabbit Basilar Artery In-vitro

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Hypocapnic constriction of the cerebral vasculature represents a major pathway by which cerebral blood flow is regulated. It is generally assumed that the constriction is mediated via the direct effect of decreased pCO_2 or elevated pH on the smooth muscle (Dacey & Duling 1982; Harder & Madden 1985; Smeda et al 1987; West et al 1992; Apkon & Boron 1995; Austin & Wray 1995; Kontos et al 1997). It is not known, however, whether release of an endothelium- or smooth-muscle-derived contracting factor (or both) may contribute to the constriction. This study investigates the mechanism underlying hypocapnic constriction of the cerebral vasculature by testing whether endothelin-1 contributes to hypocapnic constriction of the rabbit basilar artery.

New Zealand White male rabbits (2·8–3·1 kg) were anaesthetized with ketamine hydrochloride (30 mg kg^{-1}, i.m.), xylazine (6 mg kg^{-1}, i.m.) and pentobarbital sodium (35 mg kg^{-1}, i.v.), and were then exsanguinated. The brain was removed and placed in ice-cold Krebs–Ringer bicarbonate solution containing (mM): 119 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, cut into 2-mm ring segments with extreme care to preserve the endothelium intact, and each segment 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) or hypocapnic Krebs–Ringer bicarbonate solution (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; mean ± s.e.m.; n = 21 and 28, for normacapnic and hypocapnic solutions respectively).

Isometric tension was recorded by placing two tungsten wires (33 μm diam.) 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) and were allowed to equilibrate for 30–40 min before addition of agonist. All Krebs–Ringer bicarbonate solutions contained 1 μM indomethacin (Sigma). Values are expressed as means ± s.e.m.; n represents the number of tissues.

Hypocapnia induced tension in only 4 of 51 tissues. Repeated washing with hypocapnic solution accelerated the development of tension, which reached a maximum of 4·6 ± 0·3 mN/mm length × 2 (mean ± s.e.m., n = 4) within 20–30 min. The number of tissues that developed spontaneous tension was not increased by repeated washing with hypocapnic solution. However, after 1–6 challenges with 40 mM KCl for 2–4 min (which induced a plateau constriction of 5·43 ± 0·12 mN/mm length × 2, n = 47), followed by hypocapnic wash, tissues remained constricted at 5·35 ± 0·12 mN/mm length × 2 (10, 17, 12, 3, 3 and 2 remained constricted at a plateau level of tension similar to that due to 40 mM KCl after 1–6 KCl challenges, respectively). Tissues exposed to normal Krebs–Ringer bicarbonate solution did not develop tension following repeated 2–4-min 40 mM KCl challenge and normocapnic wash.

Papaverine (30 μM; Sigma) and normocapnic solution reversed the hypocapnic tension within 10–30 s and 10–30 min, respectively (Table 1). The endothelin $ET_{A/B}$-receptor antagonist, PD145065 (1 μM; Ac-D-Bhg-L-Leu-Asp-L-Ile-L-Ile-L-Trp; Parke Davis Pharmaceutical, gift) relaxed 7 tissues (Table 1), while 1 tissue did not relax over the time period during which plateau

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Table 1. Relaxation of hypocapnic constriction of rabbit basilar artery. Tension was induced in rabbit basilar artery rings following hypocapnia and, in some cases, repeated exposure to 40 mM KCl. Constricted tissues were then challenged with normocapnia, 30 μM papaverine, 1 μM PDI45065 or 1 μM BQ610.

	n	% Relaxation
Normocapnia	6	99·4 ± 1·3
Papaverine	2	102·4
PD145065	7	97·1 ± 2·3
BQ610	10	96·2 ± 3·7

Results are expressed as percent relaxation of the tension.

tension of control tissues was maintained (2–2·5 h). The ET_A-receptor antagonist, BQ610 (1 μM; homopiperidinyl-CO-Leu-D-Trp(CHO)-D-Trp; Peptides International) also relaxed 10 of 13 hypocapnic constricted tissues (Table 1), while subsequent addition of 10 μM BQ610 completely relaxed 2 of the remaining 3 tissues. Hypocapnic tension was restored to the former level when tissues relaxed with BQ610, PD145065, papaverine or normocapnic solution, were washed with hypocapnic solution. Phosphoramidon (Peptides International) variably affected hypocapnic tension, in that 0·1–0·2 mM phosphoramidon did not always relax the constriction over the time period during which plateau tension of control tissues was maintained.

Exposure of hypocapnic tissue to 3 nM endothelin-1 also resulted in a plateau constriction which was maintained even after repeated hypocapnic washes (5·3 mN/mm length × 2; n = 2). BQ610 (1 μM) relaxed the tension by 97·6% (n = 2). In normocapnic solution, 3 nM endothelin-1 elicited 25·2 ± 7·9% of the maximal endothelin-1 constriction (6·22 ± 0·27 mN/mm length × 2; means ± s.e.m.; n = 11).

This study suggests that hypocapnic constriction of the cerebral vasculature may be endothelin-1 mediated. This conclusion is based on two findings. Firstly, PD145065 and BQ610, endothelin $ET_{A/B}$- and endothelin ET_A-receptor antagonists, respectively, relaxed hypocapnic-constricted rabbit basilar artery. Secondly, a relatively low endothelin-1 concentration (3 nM) triggered subsequent constriction that was relaxed by BQ610. Although the pO_2 of the hypocapnic solution (291 mmHg) was less than that of the normocapnic solution, this level of pO_2 is still relatively high and would not be expected to cause constriction.

The endothelin-1-dependence of the hypocapnic constriction could result from increased endothelin-1 release or from increased contractile sensitivity to endothelin-1. In support of the former possibility is the observation that endothelin receptor antagonists do not decrease basal tone of the rabbit basilar artery in-vitro under normocapnic conditions (unpublished observations), suggesting that the amount of basal endothelin-1 release is minimal. While the inability of phosphoramidon to consistently relax the hypocapnic constriction may argue against the suggestion that increased endothelin-1 release underlies the constriction, it should also be considered that the constriction may be dependent on the release of stored, rather than newly synthesized, endothelin-1.

The mechanism whereby KCl challenge presumably triggers the initial release of endothelin-1 and, thus, the early development of hypocapnic constriction, is not clear. However, it is likely that the further development, as well as maintenance, of the hypocapnic constriction results from endothelin-1-induced endothelin-1 release, since initial endothelin-1 challenge also triggered subsequent endothelin-1-dependent hypocapnic tension. In this regard, we proposed previously that the spasm following subarachnoid haemorrhage was maintained via endothelin-1-induced endothelin-1 release (Zuccarello et al 1998). Clearly, measurements of endothelin-1 release would assist in determining the relative involvement of endothelin-1 release and increased endothelin-1 contractile sensitivity in hypocapnic constriction, and also the cellular source of the endothelin-1 (i.e., smooth muscle or endothelium, or both).

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