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Effect of hydroxocobalamin on vasodilatations to nitrergic transmitter, nitric oxide and endothelium-derived relaxing factor in guinea-pig basilar artery

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Abstract

In endothelium-denuded guinea-pig isolated basilar artery preparations, hydroxocobalamin (30, 100 and 300 μM) concentration-dependently inhibited the vasodilator responses to exogenous nitric oxide (NO), whereas the vasodilator responses to nitrergic nerve stimulation were slightly reduced by high (100 and 300 μM) but not by the low (30 μM) concentration of hydroxocobalamin. Vasodilatation in response to sodium nitroprusside (10–100 nM) was totally abolished by 300 μM hydroxocobalamin. In endothelium-intact preparations, vasodilator responses to acetylcholine (0.3–3 μM) were significantly reduced or abolished by hydroxocobalamin (30–300 μM). The mean reduction by hydroxocobalamin of relaxations to acetylcholine was significantly greater than that of the equivalent response evoked by nitrergic nerve stimulation. The findings suggest that the nitrergic transmitter in the guinea-pig basilar artery may be quantitatively less susceptible than the endothelium-derived relaxing factor to the NO scavenger hydroxocobalamin. © 1997 Elsevier Science B.V.

Keywords: Basilar artery; EDRF (endothelium-derived relaxing factor); Endothelium-denuded; (Guinea-pig); Hydroxocobalamin; Nitrergic transmitter; Nitric oxide (NO); Vasodilatation

1. Introduction

It has been well established that inhibitory non-adrenergic, non-cholinergic neurotransmission at certain neuroeffector junctions is dependent on the activity of nitric oxide synthase, which forms nitric oxide (NO) from L-arginine: this process is termed nitrergic transmission (for review see Rand and Li, 1995a). However, the exact nature of the nitrergic transmitter has not been elucidated. Some evidence suggests that the mediator of nitrergic transmission is a NO-releasing substance rather than NO itself because of the fact that, in a number of tissues, various agents that inactivate NO and thereby block the response to exogenous NO fail to block the response to nitrergic nerve stimulation (Hobbs et al., 1991; Barbier and Lefebvre, 1992; Knudsen et al., 1992; Gibson et al., 1994; Rand and Li, 1995b).

One such agent having this differential activity is hydroxocobalamin, which interacts with NO to form a not clearly understood NO-adduct (Rochelle et al., 1995). The interaction of hydroxocobalamin with NO has been used to investigate the nature of the nitrergic transmitter. Thus, Rajanayagam et al. (1993) showed that hydroxocobalamin in concentrations which blocked NO-induced and endothelium-derived relaxing factor (EDRF)-mediated relaxations in the rat aorta, also blocked NO-induced relaxations in the rat anococcygeus muscle, but failed to affect the relaxation elicited by the nitrergic nerve stimulation, suggesting a difference between the nitrergic transmitter on the one hand and EDRF and NO on the other hand. The differential effect of hydroxocobalamin in rat anococcygeus muscle was confirmed (Li and Rand, 1993; La et al., 1996) and has also been demonstrated in rat gastric fundus strips (Jenkinson et al., 1995). However, hydroxocobalamin has been shown to block responses to the nitrergic transmitter in the bovine retractor penis (Paisley and Martin, 1996)

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and mouse anococcygeus muscle (Lilley and Gibson, 1996), and in the latter tissue it equally blocked relaxations elicited by exogenous NO. Thus, the variant activity of hydroxocobalamin in different tissues weakens the evidence for the hypothesis that the nitrergic transmitter is not NO but may be a NO-releasing compound (Lilley and Gibson, 1996).

Cerebral arteries from many animal species, including humans, have a nitrergic vasodilator innervation (Toda and Okamura, 1990; Lee and Sarwinski, 1991; Toda, 1993) and also exhibit EDRF-mediated vasodilatation, and both have been demonstrated in guinea-pig isolated basilar artery preparations (Jiang et al., 1997). Consequently, this tissue provides the opportunity to compare the effect of hydroxocobalamin on relaxations induced by nitrergic nerve stimulation to those induced by EDRF in a single tissue. Therefore, the present study with guinea-pig isolated basilar artery preparations was carried out to investigate the effects of hydroxocobalamin on vasodilator responses to the nitrergic transmitter, EDRF and exogenous applied NO.

2. Methods

2.1. Basilar artery preparations

The procedure for isolating and setting up preparations of the basilar artery were as described previously (Jiang et al., 1997). Briefly, guinea-pigs (350–450 g) of either sex were treated with heparin (1000 U kg^{-1}, i.p.), anaesthetized with pentobarbitone sodium (30 mg kg^{-1}, i.p.) and were killed by decapitation. The brain, including the brain stem, was removed into physiological salt solution at room temperature. The basilar artery was identified and a 2 mm long segment with an external diameter of 400–500 μm was dissected out and mounted in a myograph of the type described by Mulvany and Halpern (1977). The preparation was perfused with fresh physiological salt solution at 37°C at 3 ml per min. Changes of isometric tension of the vessel wall was recorded by a Rikadenki pen oscillograph.

In order to eliminate the involvement of EDRF in nerve stimulation-induced responses (Jiang et al., 1997), we used endothelium-denuded preparations to study the nerve stimulation-induced vasodilatation. The endothelium was denuded by perfusing 1:1000 Triton X-100 solution in physiological salt solution intraluminally for 1 min through a fine cannula before the vessel was dissected out from the brain. Removal of the endothelium was confirmed by abolition of the relaxation in response to 3 μM acetylcholine.

2.2. Experimental procedures

Before experiment interventions were commenced, the preparation was allowed an equilibration period of 30 min, then the resting tension was adjusted to 3 mN and a further 60 min equilibration period was given. Relaxant responses were observed after the active tension was raised by 1 μM prostaglandin $F_{2\alpha}$.

Nitrergic nerves were activated by electrical field stimulation (0.2 ms pulses at 10 Hz for 30 s) applied through a pair of electrodes set parallel to the vessel segment. Saturated NO solution was injected into the perfusing physiological salt solution by a microsyringe. Other drugs were also administered through the perfusing physiological salt solution. All electrical stimulation-, NO- and sodium nitroprusside-induced responses were studied in endothelium-denuded preparations. At the beginning of each session of experimentation, either electrical field stimulation or a vasodilator drug was repeatedly applied until stable responses were obtained. After each session, the preparation was washed with fresh physiological salt solution for at least 20 min before further experimentation.

EDRF-mediated responses in endothelium-intact preparations were induced by acetylcholine. After control responses to acetylcholine had been established, the tissue was exposed to hydroxocobalamin at different concentrations and the responses to acetylcholine were repeated.

2.3. Drugs and solution

The drugs used and their provenance is as follows: acetylcholine chloride (Sigma), heparin (CSL, Australia), hydroxocobalamin hydrochloride (Sigma), pentobarbitone sodium (Boehringer Ingelheim, Australia), prostaglandin $F_{2\alpha}$ (tris salt, Sigma), sodium nitroprusside (Sigma), Triton X-100 (Sigma). Saturated NO aqueous solution (2 mM) was prepared as previously described by Rajanayagam et al. (1993), using compressed NO gas (CIG, Melbourne).

The physiological salt solution had the following composition (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.14.

2.4. Results and statistics

The results were expressed as percentage of the precontraction produced by 1 μM prostaglandin $F_{2\alpha}$. Values were mean $\pm$ standard error of the mean (S.E.M.). Paired or unpaired *t*-test and one-way analysis of variance (One-Way ANOVA) were used; $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Effect of hydroxocobalamin on the prostaglandin $F_{2\alpha}$-induced active tone

In the guinea-pig basilar artery preparations with intact endothelium, hydroxocobalamin at 100 and 300 μM sig-

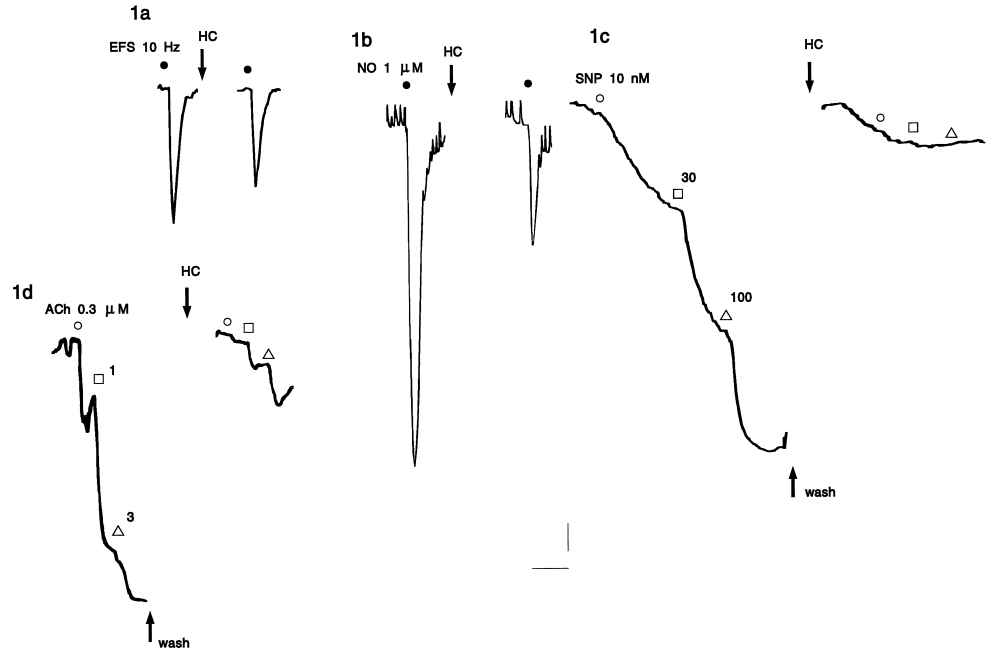

Fig. 1. Typical traces showing the inhibitory effect of hydroxocobalamin (300 μM) in isolated guinea-pig basilar arteries on the vasodilatations induced by electrical field stimulation (a), NO (b), sodium nitroprusside (c) in endothelium-denuded preparations and acetylcholine (d) in endothelium-intact preparations. HC: hydroxocobalamin (300 μM); the vertical bar represents 2 mN; the horizontal bar represents 5 min.

nificantly enhanced the contractile response to prostaglandin $F_{2\alpha}$ (1 μM). The mean contraction before and in the presence of 100 and 300 μM hydroxocobalamin were 23.2 mN $\pm$ 1.78, 25.7 mN $\pm$ 1.71* and 25.7 mN $\pm$ 1.47* respectively ($n = 9$, $^{*}P < 0.05$, paired t-test, compared with control response). However, in endothelium-denuded preparations, hydroxocobalamin up to 300 μM did not affect the active tone induced by prostaglandin $F_{2\alpha}$.

3.2. Electrical field stimulation-induced vasodilatation

A typical trace illustrating the effect of 300 μM hydroxocobalamin on the electrical field stimulation-induced

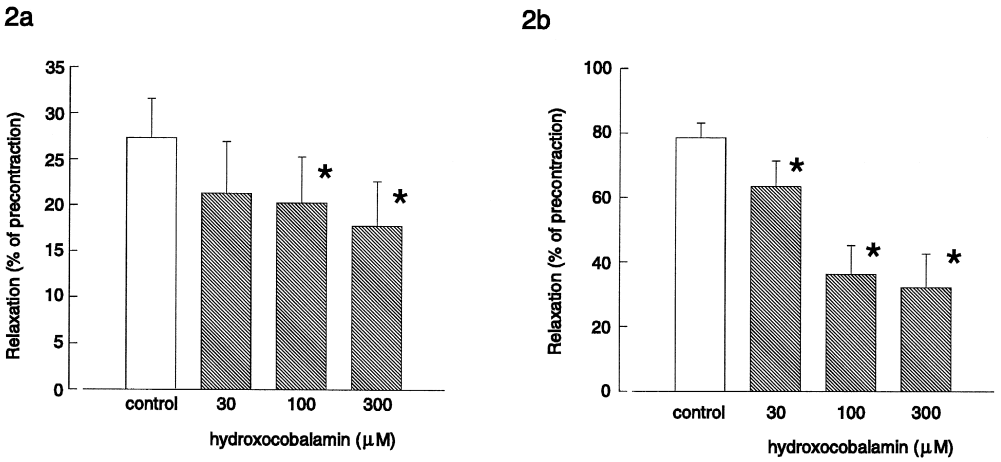

Fig. 2. Effect of hydroxocobalamin on the vasodilator responses elicited by electrical field stimulation (a, $n = 6$) and 1 μM NO (b, $n = 5$). Responses are expressed as percentages of the precontraction produced by 1 μM prostaglandin F_{2a}. The open columns represent mean of the control responses; the closed columns represent mean of the responses after treatment with hydroxocobalamin at the concentration indicated below. Vertical bars are S.E.M. $^{*}P < 0.05$, paired t-test, compared with control.

vasodilator response is shown in Fig. 1a. In endothelium-denuded basilar artery preparations, hydroxocobalamin at 100 and 300 μM significantly reduced the vasodilatation elicited by electrical field stimulation (10 Hz), whereas the response to electrical field stimulation was not significantly affected by 30 μM hydroxocobalamin (Fig. 2a). The mean reductions of the electrical field stimulation-induced vasodilator responses produced by hydroxocobalamin (100 and 300 μM), expressed as percentages of the pre-hydroxocobalamin control response, were 31.8% $\pm$ 10.42 and 42.4% $\pm$ 12.84 ($n = 6$), respectively.

3.3. NO- and sodium nitroprusside-induced vasodilatations

NO was applied only in one concentration of 1 μM because the responses to NO in lower concentrations were variable in the present experimental system. Hydroxocobalamin (30–300 μM) markedly reduced the vasodilator response to exogenous NO in a concentration-dependent manner: the mean data are shown in Fig. 2b. A typical trace of the inhibitory effect of 300 μM hydroxocobalamin on NO-induced vasodilatation is shown in Fig. 1b. The mean reductions of NO-induced vasodilatations produced by hydroxocobalamin (100 and 300 μM), expressed as percentages of the control response, were 55.4% $\pm$ 10.65 and 57.8% $\pm$ 13.2 ($n = 5$), respectively.

Hydroxocobalamin (300 μM) totally abolished the vasodilator response to sodium nitroprusside (10–100 nM) (Fig. 1c).

3.4. Acetylcholine-induced vasodilatation

In endothelium-intact preparations, acetylcholine (0.3–3 μM) elicited concentration-dependent vasodilatations. Hydroxocobalamin (30–300 μM) markedly reduced the acetylcholine-induced vasodilator responses as shown in Fig. 1d and the mean data are summarized in Fig. 3.

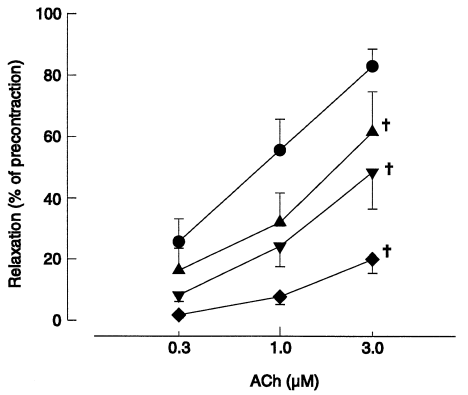

Fig. 3. Effect of hydroxocobalamin (30–300 μM) on the vasodilator responses produced by acetylcholine (0.3–3 μM). (●) control; (▲) hydroxocobalamin 30 μM; (▼) hydroxocobalamin 100 μM; (♦) hydroxocobalamin 300 μM. Responses are presented as percentages of the precontraction produced by 1 μM prostaglandin F_{2a}. Each point represents the mean of the responses from 4 experiments. Vertical bars are S.E.M. $^{\dagger}P < 0.05$, One-Way ANOVA followed by Student's *t*-test, compared with control response.

The vasodilator response to 0.3 μM acetylcholine was about the same magnitude as that elicited by electrical field stimulation (10 Hz). The mean reduction produced by 300 μM hydroxocobalamin of the vasodilator response to 0.3 μM acetylcholine was 95.2% $\pm$ 2.89 ($n = 4$, expressed as percentage reduction of control response), which is significantly different from that of electrical field stimulation-induced vasodilator response (42.4% $\pm$ 12.8, $n = 6$, $P < 0.05$, unpaired *t*-test). In contrast, there is no statistically significant difference between the mean reductions produced by hydroxocobalamin (30–300 μM) of relaxations with similar amplitudes induced by NO (1 μM) and ACh (3 μM).

4. Discussion

Hydroxocobalamin (vitamin B_{12a}) has a cobalt-containing corrin core in the molecule. Recent studies indicate that it can interact with NO to form a novel, not precisely defined complex in which NO is bonded to the hydroxocobalamin molecule (Rochelle et al., 1995). Such binding is reversible since this complex is capable of transferring the NO moiety, in form of nitrosonium (NO^+), to other NO-binding substances accompanied by a reduction of hydroxocobalamin to a reduced form (vitamin B_{12r}) (Brouwer et al., 1996).

The use of hydroxocobalamin as a NO scavenger to study nitrergic mechanisms was initially carried out in the rat anococcygeus muscle and later was extended to other gastrointestinal and urogenital tissues (Li and Rand, 1993; De Man et al., 1995; Jenkinson et al., 1995; Lefebvre, 1996; Lilley and Gibson, 1996; Paisley and Martin, 1996). As far as we know this is the first report on the effect of hydroxocobalamin on nitrergic neurotransmission in a vascular tissue. The finding that hydroxocobalamin reduced the vasodilatations induced by both NO and nitrergic nerve stimulation in the guinea-pig basilar artery is similar to the results obtained in the mouse anococcygeus muscle (Lilley and Gibson, 1996) and the bovine retractor penis (Paisley and Martin, 1996), but is in contrast with the findings in the rat anococcygeus muscle (Li and Rand, 1993; La et al., 1996), the rat gastric fundus (Jenkinson et al., 1995; Lefebvre, 1996) and the dog ileocolonic junction (De Man et al., 1995), in which hydroxocobalamin in concentrations that considerably reduced the relaxant responses to NO had little effect on nitrergic transmission. There are several possibilities which may explain the difference between tissues in regard to the differential actions of hydroxocobalamin. First, the nitrergic transmitter may differ between different species and/of different tissues, although there is no convincing evidence to support this at present time. Alternatively, there are tissue factors which may interfere with the actions of hydroxocobalamin and the contribution of those factors may be varied between tissues. It has been suggested that endogenous superoxide

dismutase and antioxidants are involved in the nitrergic transmission (Martin et al., 1994; Lilley and Gibson, 1995, 1996; Lefebvre, 1996; Paisley and Martin, 1996), although neither superoxide dismutase inhibitor nor antioxidants were found to affect the inhibition of nitrergic responses by hydroxocobalamin (Lefebvre, 1996; Paisley and Martin, 1996; Li et al., unpublished results).

The finding that hydroxocobalamin strongly inhibited EDRF-mediated vasodilatations induced by acetylcholine in the guinea pig basilar artery is similar to the earlier finding in the rat aorta (Rajanayagam et al., 1993). Also, hydroxocobalamin significantly enhanced the contractile responses to prostaglandin $F_{2\alpha}$ in the endothelium-intact preparations but not in the endothelium-denuded preparations. Such results indicate that EDRF, either released under basal conditions or released by agonists, is as readily blocked by hydroxocobalamin as is free NO. On the other hand, hydroxocobalamin was less effective against the vasodilatation induced by nitrergic nerve stimulation, since the mean reduction by hydroxocobalamin of the relaxation to acetylcholine was significantly greater than that of the equivalent relaxation to nitrergic nerve stimulation. Since this result was obtained in the same tissue, it is unlikely that tissue factors are involved in this differential effect of hydroxocobalamin. However, such difference appears to be quantitative rather than qualitative as higher concentration of hydroxocobalamin did reduce responses to both acetylcholine and nitrergic nerve stimulation. This differential effect of hydroxocobalamin may be tentatively interpreted as evidence that the nitrergic transmitter differed from EDRF; however, more experiments are necessary to establish the role of other factors such as accessibility to the tissue targets by this agent. It should be noted that there is evidence for the view that the nitrergic transmitter is more likely to be a NO adduct rather than free NO (Rand and Li, 1995c).

Vasodilator responses to the NO donor sodium nitroprusside were more readily blocked by hydroxocobalamin than those to exogenous NO. Similar findings were obtained earlier in the rat anococcygeus muscle (Li and Rand, 1993). It has been suggested that sodium nitroprusside exerts its vasodilating activity after biotransformation resulting in liberation of NO (Marks et al., 1991). The site of biotransformation is believed to be subcellular membrane fractions of smooth muscle cells, but the exact mechanism is still not elucidated (Kowaluk et al., 1992). It is not clear whether the inhibition by hydroxocobalamin of the sodium nitroprusside-induced response involves interference with the biotransformation of sodium nitroprusside as well as sequestration of NO. Further experiments are necessary before a conclusion can be drawn.

In summary, in guinea-pig isolated basilar artery preparations, the NO scavenger hydroxocobalamin inhibits vasodilator responses to electrical nerve stimulation, acetylcholine and exogenous NO. The mean reduction produced by hydroxocobalamin of relaxations to acetylcholine was significantly greater than that of the equivalent response evoked by nitrergic nerve stimulation. It is suggested that the nitrergic transmitter in the guinea-pig basilar artery is less susceptible than the endothelium-derived relaxing factor to hydroxocobalamin.

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