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Effects of hydroxocobalamin on nitrergic transmission in rat anococcygeus and bovine retractor penis muscles: sensitivity to light

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Abstract

Hydroxocobalamin inhibited nitrergic nerve-induced relaxations in rat anococcygeus and bovine retractor penis muscles in a concentration-dependent manner. In the rat anococcygeus muscle, the inhibition was greater in light than in dark conditions, whereas in the bovine retractor penis the inhibition was similar under both conditions.

Keywords: Hydroxocobalamin; Nitrergic transmission; Light sensitivity

A number of agents block responses to nitric oxide (NO) in aqueous solution but fail to block responses to the nitrergic transmitter in some tissue (Rand and Li, 1995). One such agent, hydroxocobalamin, binds NO (Kaczka et al., 1951) by a complex set of reactions (Rochelle et al., 1995) and abolished responses to NO at a concentration (100 μM) that was reported to have no effect on relaxations induced by nitrergic nerves in the rat anococcygeus muscle (Rajanayagam et al., 1993; La et al., 1996). At a recent symposium (Reid et al., 1996), Martin reported that hydroxocobalamin (10 μM–1000 μM) produced concentration-dependent reductions of nitrergic nerve stimulation-induced relaxations of the rat anococcygeus muscle. The discrepancy between this and the previous findings by Rajanayagam et al. (1993) and La et al. (1996), at least as far as the concentration of 100 μM in the rat anococcygeus muscle is concerned, led to this collaborative study to determine whether methodological differences accounted for it. In a systematic examination of identified differences, the one that emerged as decisive was the presence or absence of light, as described here. Furthermore, since nitrergic transmission in the bovine retractor penis muscle was inhibited by hydroxocobalamin in the light (Paisley and Martin, 1996), this was tested in the absence of light.

Rat anococcygeus and bovine retractor penis muscles were isolated and set up as described by Paisley and Martin (1996). Field stimulation (FS: 4 Hz, 10 s) was delivered from a Grass S88 stimulator at a pulse width of 0.5 ms at supramaximal voltage. Noradrenergic contractile responses to FS were blocked and the tone raised with guanethedine (30 μM), whereupon FS produced relaxations mediated by the nitrergic transmitter. Experiments in the light were performed under normal conditions of room lighting. Experiments in the dark were conducted in a photographic dark room under red light.

The relaxant responses to FS in each tissue, in g decrease in tension, did not differ significantly between light and dark conditions ($P > 0.05$, unpaired *t*-test): in rat anococcygeus muscles, the relaxations were 3.3 ± 0.2 ($n = 10$) and 3.4 ± 0.2 ($n = 12$), respectively; in bovine retractor penis muscles, the relaxations were 3.9 ± 0.3 ($n = 8$) and 3.5 ± 0.3 ($n = 8$), respectively.

Hydroxocobalamin inhibited nitrergic nerve stimulation-induced relaxations in a concentration-dependent manner in both muscles. In rat anococcygeus muscles, the inhibition was greater in light than in dark conditions, amounting to an apparent 3-fold increase in potency in terms of a shift of the curve to the left in the light (Fig. 1A). However, in the bovine retractor penis, hydroxocobalamin had the same inhibitory effect under both conditions ($P < 0.05$, ANOVA) (Fig. 1B).

The effect of light on the degree of inhibition by hydroxocobalamin of nitrergic nerve stimulation-induced

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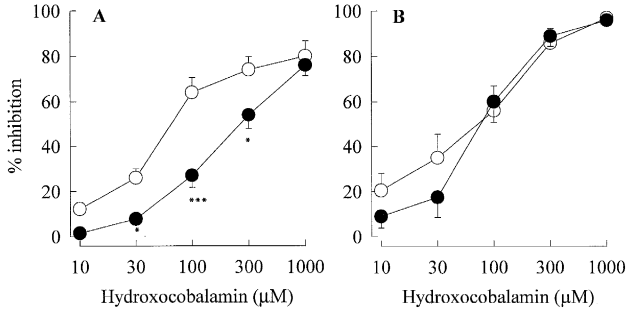

Fig. 1. Inhibition by hydroxocobalamin of relaxations of rat anococcygeus (A) and bovine retractor penis (B) muscle following field stimulation in the light (○) and dark (●). Inhibition is expressed as percentage reduction of the responses in the absence of hydroxocobalamin. * $P < 0.05$, *** $P < 0.001$.

relaxations of anococcygeus muscles largely accounts for the discrepancy that triggered this report, since the experiments of Rajanayagam et al. (1993) and La et al. (1996) were carried out in dark conditions because hydroxocobalamin is labelled as light-sensitive.

The present finding that 100 μM hydroxocobalamin produced a 27% reduction of the response to nitrergic nerve stimulation in the rat anococcygeus in dark conditions requires re-evaluation of the earlier conclusions of Rajanayagam et al. (1993) and La et al. (1996) that the transmitter differs from NO, which was based on their findings that 100 μM hydroxocobalamin had little effect. Thus, the difference between NO and the transmitter is quantitative rather than qualitative, the former being blocked more readily than the latter. Although it is still possible that the transmitter is chemically distinct from NO, the difference in diffusional distance between the transmitter and exogenous NO could explain the finding as proposed by Wood and Garthwaite (1994).

It is unlikely that the mechanism by which light potentiates the action of hydroxocobalamin on nitrergic transmission in rat anococcygeus muscles is due to a photolytic change in hydroxocobalamin since light does not affect its action on nitrergic transmission in the bovine retractor penis. It is possible that light makes the nitrergic transmitter in the rat anococcygeus more reactive with hydroxocobalamin, in which case it would follow that the transmission process in the bovine retractor penis differs in that it is not affected by light; however, light would penetrate more readily through the thin, translucent tissue of the anococcygeus than through the considerably thicker opaque tissue of the bovine retractor penis.

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