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Rapid communication

Cardiovascular effects produced by systemic injections of nitro blue tetrazolium in the rat

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Nitro blue tetrazolium is a powerful electron acceptor which is widely used to localize NADPH-dependent flavin-containing enzymes known as NADPH diaphorases. By accepting electrons, nitro blue tetrazolium is known to inhibit the activity of these enzymes. The present study examined the effects of intravenous nitro blue tetrazolium on arterial blood pressure and regional blood flows in urethane-anesthetized rats. Nitro blue tetrazolium (0.1–5 mol/kg) produced a dose-dependent hypotension and differential effects on regional hemodynamics including decreases in hindquarter and mesenteric vascular resistances and a marked increase in renal resistance. These results demonstrate that systemic administration of nitro blue tetrazolium produces profound hemodynamic effects, the mechanisms of which remain to be elucidated.

NADPH diaphorase; Nitro blue tetrazolium; Haemodynamics; (Rat)

There is now considerable evidence that nitric oxide (NO), or NO factors such as the nitrosothiol S-nitrosocysteine, are critically involved in the regulation of vascular tone (Moncada et al., 1991). A recent study has provided evidence that neuronal NADPH diaphorase is an isoform of NO synthase (Hope et al., 1991). The localization of NADPH diaphorase-containing cells involves the incubation of tissues with nitro blue tetrazolium, whereby this electron-accepting compound is reduced to an insoluble blue diformazan in an NADPH-dependent manner (Scherer-Singler, 1983). In the periphery, nitro blue tetrazolium staining is selective for certain populations of cells, including autonomic and sensory neurons, whereas macrophages and endothelial cells are apparently not stained (Hope et al., 1991).

Nitro blue tetrazolium prevents the electron transfer involved in processing the endogenous substrate for NADPH diaphorase, and biochemical studies have demonstrated that nitro blue tetrazolium competitively inhibits NO synthase (Hope et al., 1991). It is well-established that NO synthesis inhibitors such as L-nitro arginine produce a sustained hypertension mediated by increases in regional vascular resistances (Moncada et al., 1991). As such, the aim of the present study was to determine the effects of systemic injections of nitro blue tetrazolium on arterial blood pressure (MAP) and regional hemodynamics in anesthetized rats. We now report that the i.v. injection of nitro blue tetrazolium produces hypotension, a vasodilation in the hindquarter and mesenteric vasculature, and a marked renal vasoconstriction. Except for the renal vasoconstriction, these effects are *not* consistent with those associated with the inhibition of NO synthase.

Male Sprague-Dawley rats (300–400 g; n = 6) were anesthetized with urethane (1 g/kg i.p.) and prepared for i.v. injection of drugs and measurement of MAP, heart rate (HR), and hindquarter, renal, and mesenteric blood flows as described previously (Lacolley et al., 1991). Data are expressed as mean ± S.E.M. The significance of differences from baselines was determined by repeated measures analysis of variance (ANOVA) followed by Student's modified t-test with the Bonferroni correction for multiple comparisons utilizing the error mean square term from the ANOVA. Data were considered significant at a 0.05 confidence level.

The i.v. injection of nitro blue tetrazolium (0.1–5.0 μmol/kg) produced a dose-dependent decrease in

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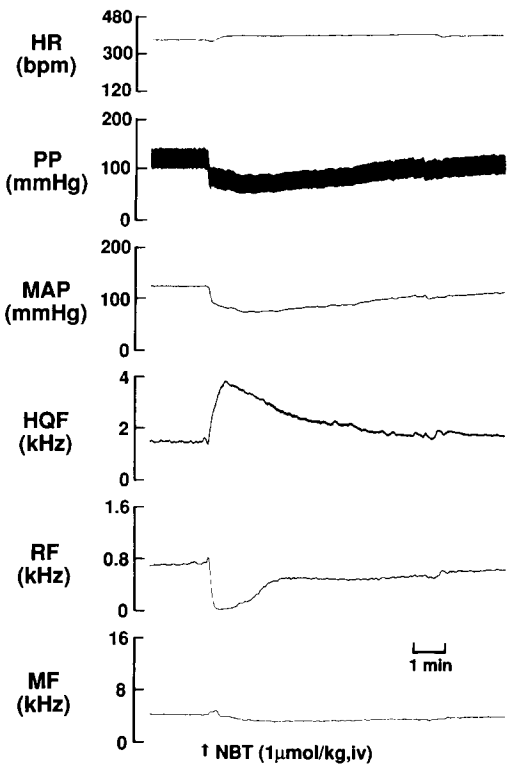

Fig. 1. A typical example of the effects of i.v. administration of nitro blue tetrazolium (NBT) (1 μmol/kg) on HR, MAP, and hindquarter (HQF), renal (RF) and mesenteric (MF) blood flows in a urethane-anesthetized rat.

MAP and differential effects on regional hemodynamics. An example of the cardiovascular effects produced by the 1 μmol/kg dose is shown in fig. 1. This dose of nitro blue tetrazolium produced an immediate (peak response within 30 s) fall in MAP (−39 ± 4%, n = 6), decreases in hindquarter (−68 ± 3%) and mesenteric (−15 ± 4%) resistances, a large increase in renal resistance (+510 ± 84%), but no effect on HR. The responses produced by this dose of nitro blue tetrazolium returned to baseline by 10 min. The maximal dose used in these studies, i.e. 5 μmol/kg, produced an immediate fall in MAP (−43 ± 5%), decreases in hindquarter (−66 ± 7%) and mesenteric (−27 ± 5%) resistances, and a large increase in renal resistance (+967 ± 55%).

The present study demonstrates that the systemic injection of nitro blue tetrazolium produces cardiovascular effects including a substantial hypotension, vasodilation in the hindquarter and mesenteric vasculature, and a marked vasoconstriction in the renal bed. Since nitro blue tetrazolium inhibits brain NADPH diaphorase/NO synthase by interfering with the electron transfer normally involved in processing this enzyme (Hope et al., 1991), it was expected that i.v. nitro blue tetrazolium would, similar to other NO synthesis inhibitors such as L-nitro arginine, produce a sustained pressor response and increases in regional vascular resistances (Lacolley et al., 1991). Except for the increase in renal resistance, the cardiovascular effects of nitro blue tetrazolium, i.e. decreases in MAP, hindquarter, and mesenteric resistances, are *not* consistent with a decrease in NO synthesis/release. Moreover, preliminary studies in our laboratory (unpublished observations) suggest that the hypotensive and vasodilator actions of NBT actually involve an *augmentation* of NO synthesis/release and these effects are mediated by an electron-accepting process. Specifically, an infusion of methylene blue (0.2 mg/kg/min i.v.), an inhibitor of soluble guanylate cyclase activation, or injection of the NO synthesis inhibitor L-nitro arginine methyl ester (25 μmol/kg i.v.) significantly reduces the cardiovascular effects of nitro blue tetrazolium. In addition, injection of dicumarol (20 μmol/kg i.v.), an agent which interferes with the ability of nitro blue tetrazolium to accept electrons from flavin-containing NADPH diaphorases, significantly attenuates both the magnitude and duration of the nitro blue tetrazolium-induced responses. Since particular populations of neuronal but *not* endothelial cells are stained by the NADPH diaphorase technique (Hope et al., 1991), it is possible that nitro blue tetrazolium may induce the release of NO factors from neurogenic stores, including sensory, autonomic (Moncada et al., 1991) and/or post-ganglionic sympathetic neurons (Davisson et al., 1992). However, since the mechanisms by which nitro blue tetrazolium produces its cardiovascular effects are not established, the possibility that nitro blue tetrazolium augments the synthesis/release of NO factors from the vascular endothelium or circulating blood cells such as neutrophils and platelets cannot be discounted.

Overall, these studies suggest that, contrary to expectation, systemically administered nitro blue tetrazolium may augment rather than inhibit NADPH diaphorase/NO synthase activity or NO factor release from vascular tissue. The mechanism(s) by which this electron-accepting agent may increase NO synthesis and/or release remains to be determined.

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