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The sensory nitrergic nature of the hepatic insulin sensitizing substance mechanism in conscious rabbits

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Abstract

Functional deterioration of sensory fibres in the anterior hepatic plexus or intraportal administration of 7-nitro indazole (1 mg/kg), a selective inhibitor of neural nitric oxide (NO) synthase, caused insulin resistance as determined by hyperinsulinaemic (100 μU/ml) euglycaemic (5.5 mmol/l) glucose clamping in chronically instrumented conscious rabbits. Intraportal nitroglycerin restored insulin sensitivity in either case. We conclude that NO of sensory neural origin plays a major role in endogenous neurogenic insulin sensitizing mechanisms.

Keywords: Capsaicin; Insulin resistance; Nitroglycerin

Post-prandial activation of nerves in the anterior hepatic plexus leads to the release of a hormone-like substance from the liver termed hepatic insulin sensitizing substance (HISS) (Lautt, 1999) that increases the sensitivity of peripheral tissues to the hypoglycaemic effect of insulin (Sadri and Lautt, 1999). The HISS mechanism is controlled by hepatic nitric oxide (NO) production, since intraportal administration of NO synthase inhibitors causes insulin resistance, and intraportal administration of NO donors restores insulin sensitivity in animals made insulin resistant by preceding NO synthase inhibition (Sadri and Lautt, 1999). However, neither the nature of HISS nor the source of NO has been identified. Since the anterior hepatic plexus comprises several capsaicin-sensitive sensory fibres (Erin et al., 2000) including nitrergic neurons (Shimamura et al., 2000), the present work was to study whether nitrergic sensory nerves were involved in the HISS mechanism.

These experiments conform to the European Community guiding principles for the care and use of experimental animals. Eighteen male New Zealand white rabbits (3–3.2 kg) underwent surgery as described previously (Szilvássy et al., 1994). Polyethylene catheters were inserted into a branch of the portal vein, two main branches of the jugular vein and the left carotid artery. The catheters were exteriorised through the neck. These lines were kept patent by filling with sodium heparin solution (100 IU/ml). To study the involvement of capsaicin-sensitive sensory neurons in the HISS mechanism, six animals underwent partial hepatic sensory denervation: Sponge slices of 3 mm length impregnated with 2% capsaicin solution were applied around the anterior hepatic plexus for 3 days. This treatment schedule causes functional deterioration of capsaicin-sensitive sensory fibres termed regional capsaicin desensitisation (Szolcsanyi, 1996). The control rabbits received solvent-impregnated sponges. Hyperinsulinaemic euglycaemic glucose clamp studies were carried out to estimate tissue sensitivity to the hypoglycaemic effect of insulin after a 7-day period of convalescence as follows. Insulin was infused at a constant rate (13 mU/kg, NOVO Nordisk, Copenhagen) intravenously over 120 min yielding plasma insulin immunoreactivity of 100 ± 5 μU/ml in the steady state (see below). Samples (0.3 ml) were taken from the arterial cannula for blood glucose concentration at 10-min intervals. Blood glucose was maintained at 5.5 ± 0.5 mmol/l by a variable rate of glucose infusion via the second venous cannula. The blood glucose stabilization period of at least 15 min was defined as steady state. Additional blood samples (0.5 ml) were taken for plasma insulin radioimmunoassay determi-

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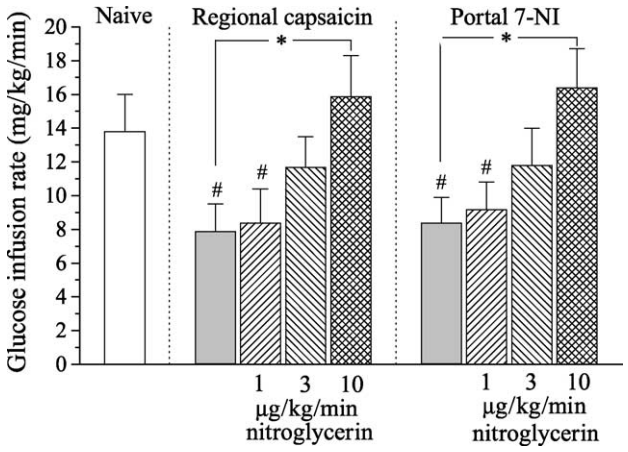

Fig. 1. Insulin resistance induced by intraportal 7-nitro indazole (1 mg/kg) or perineurial capsaicin desensitisation of the anterior hepatic plexus in conscious rabbits. Reversal by intraportal nitroglycerin. The results are means ± standard error of the mean obtained with six rabbits per group. #: significantly different from "naive" at $P < 0.05$; *: as indicated at $P < 0.05$ (analysis of variance followed by Bonferroni's *t*-test for repeated measurements).

nation at 5-min intervals during steady state. The glucose infusion rate was used to characterize insulin sensitivity (DeFronzo et al., 1979). The portal catheter was used for 7-nitro indazole (1 mg/kg single dose, Sigma, St Louis, MO) or nitroglycerin infusion. Six of the 24 chronically instrumented rabbits remained "naive", six were given intraportal 7-nitro indazole 10 min before clamping, six underwent partial hepatic sensory desensitization, and another six were equipped with placebo sponges.

Both perineurial capsaicin desensitisation and intraportal 7-nitro indazole significantly decreased insulin sensitivity. Intraportal nitroglycerin infusion restored insulin sensitivity in either case (Fig. 1). Placebo sponges were without effect (not shown).

That capsaicin-sensitive sensory nerve fibres are involved in the HISS mechanism is the major original finding of our study. Moreover, our results demonstrate that the NO involved in this mechanism is possibly of neural origin. Since both partial hepatic functional sensory denervation by perineurial capsaicin (Szolcsanyi, 1996) and low-dose intraportal application of a neural NO synthase inhibitor caused similar degree of insulin resistance reversible by intraportal nitroglycerin, NO of sensory neural origin seems to be an important trigger or mediator of the HISS mechanism. A similarly low dose of a non-selective NO synthase inhibitor was found to block hepatic NO production by Sadri and Lutt (1999). Considering that the effector function of sensory nerves is based on the release of neuropeptides (Szolcsanyi, 1996), it is possible that HISS is identical to a sensory neurotransmitter with hormone-like activity in the release of which neural NO is of crucial importance. Recent results by Oroszi et al. (1999) provided evidence for such an example in the heart, i.e. the release of the sensory neuropeptide calcitonine gene-related peptide was found to be regulated by NO.

In summary, the present results shed light on a newly recognized feature of the effector function of sensory nerves. Besides theoretical interests, the results may deserve some innovative aspects for NO donors as insulin sensitizers.

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