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INSULIN INHIBITS NOREPINEPHRINE OVERFLOW FROM PERIPHERAL SYMPATHETIC NERVE ENDING

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Summary: The effects of insulin on peripheral nervous system are unknown. We therefore studied the effects of insulin on sympathetic nerve activity in isolated mesenteric arteries of Sprague-Dawley rats. The overflow of norepinephrine (NE) by electrical stimulation was used as the index of sympathetic nervous system activity. Insulin (0.5 to 1U/l) decreased the NE release in a dose-dependent fashion. This inhibitory effect was, however, reversed by either 5×10^{-5} M cocaine or 5×10^{-4} M ouabain treatment. Thus, we postulate that insulin attenuates NE overflow from peripheral sympathetic nerve endings, probably due to enhanced NE reuptake. © 1992 Academic Press, Inc.

Norepinephrine (NE) release is influenced not only by reflexes via the central nervous system but also by local action of modulators in peripheral nerve endings. It has been demonstrated that systemic administration of insulin increases plasma NE level in humans (1), although the precise mechanism is unknown. This increased plasma NE by systemic insulin administration may be due to insulin effects on the central or peripheral systems. This study was therefore performed to determine the effect of insulin on NE release from peripheral nerve endings in the mesenteric arteries of rats.

Materials and Methods

The mesenteric arteries of 8 to 9 week-old male Sprague-Dawley rats were isolated by a modification of Castellucci's method (2). The intestine was discarded and mesenteric arteries were promptly connected to the perfusion apparatus. The preparations were perfused with Krebs-Henseleit solution with 400mg/l of bovine serum albumin by use of a peristaltic pump (3ml/min). Constituents of this solution were as follows (mmol/l): NaCl, 114.5; KCl, 4.6; KH_2PO_4, 1.4; $MgSO_4$, 2.4; $CaCl_2$, 2.5; $NaHCO_3$, 25; Glucose 5.6. The solution was continuously oxygenated with a gas mixture of 95% O_2-5% CO_2 at 37°C.

Platinum electrodes placed around the peri-arterial plexi of the mesenteric arteries were used to stimulate the sympathetic nerves. A standard

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electrical stimulus (8 Hz, 60s duration every 15 min) was given. Two stimuli were applied with each buffer and a total of six stimuli were applied to each preparation. A 30 min equilibration period was allowed prior to each experiment.

Exogenous norepinephrine (Sigma Chemical Co., St Louis MO) in a dose of 0.1mmol was injected as a bolus via a microinjector after 15 min of stabilization in the presence of human insulin (Shionogi Pharmaceutical Co., Osaka, Japan)(0.25U/l, 0.5U/l, and 1U/l). The change of perfusion pressure was also recorded by a pressure transducer (model TP-200T; Nihon Kohden, Tokyo, Japan) connected to a thermal array recorder (model WS-641G; Nihon Kohden).

The perfusate from the mesenteric vascular preparation was collected into tubes containing 10 mg EDTA and subjected to high performance liquid chromatography for the measurement of NE (3). The samples were collected every two min during and after nerve stimulation. NE overflow did not change significantly after at least 10 consecutive electrical stimulations (data not shown). The overflow of endogenous NE and the pressor response induced by electrical stimulation were completely abolished by the addition of guanethidine to the perfusate (data not shown), indicating that they reflects the activity of sympathetic nerves. NE overflow was also measured in the presence of human insulin (Shionogi Pharmaceutical Co., Osaka, Japan) (0.25U/l, 0.5U/l, and 1U/l), cocaine (Takeda Chemicals Co., Osaka, Japan) (5×10^{-5}M), ouabain (Sigma Chemical Co., St Louis MO) (5×10^{-4}M) or a combination of these agents. The nerves were incubated in these agents 15 min before nerve stimulation. NE overflow was defined as perfusate NE content per wet tissue weight.

Values are presented as means ± SD. Statistical significance was determined by paired and unpaired (comparison of percent changes) t-test. A p value of <0.05 was considered to indicate a significant difference.

Results

Effect of exogenous norepinephrine

The percent change of perfusion pressure to bolus injection of exogenous norepinephrine was not attenuated by insulin in doses of 0.25, 0.5 and 1U/l: -3.3±1.4 (ns, n=4),+0.6±2.5 (ns, n=4), -0.3±4.3%, (ns, n=4), respectively.

Effect of insulin on NE overflow

No differences were seen in the basal overflow of NE without or with insulin at the doses of 0.25, 0.5 or 1U/l (0.044±0.028, 0.053±0.016, 0.042±0.024, 0.043±0.033ng/g respectively, n=5) and basic perfusion pressure (data not shown). Electrical stimulation increased NE overflow (0.517±0.169ng/g, $p<0.01$, n=5), but was attenuated by insulin in a dose-dependent fashion (0.442±0.151, 0.176±0.047, 0.147±0.072ng/g; ns, $p<0.01$, $p<0.01$; 0.25, 0.5 and 1U/l insulin, respectively, n=5) (Fig. 1).

Effect of cocaine

5×10^{-5}M cocaine alone increased NE overflow (0.883±0.135 vs 0.647±0.179ng/g, $p<0.01$, n=6). The additional administration of 1U/l insulin to

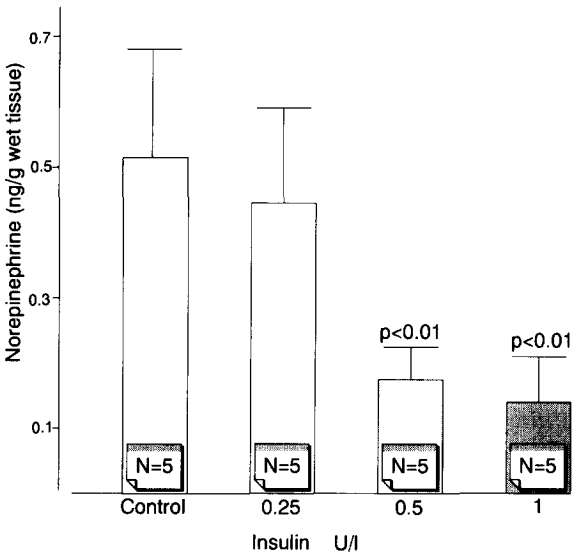

Figure 1. Effect of insulin on electrically stimulated norepinephrine overflow from peripheral sympathetic nerves.
Mesenteric arteries were isolated with the accompanying nerve plexi, preincubated with insulin, and norepinephrine overflow was assayed as described in Materials and Methods.
means±SD.
N stands for the number of rats.

the cocaine-treated mesenteric arteries failed to decrease NE overflow (0.777±0.201 vs. 0.883±0.135ng/g, n.s., n=6) (Fig. 2 A). Moreover, the addition of cocaine to insulin-treated arteries was able to reverse the attenuation of NE overflow by 1U/l insulin (0.374±0.212ng/g, insulin vs. 0.543±0.115ng/g, insulin and cocaine, $p<0.01$, n=5) (Fig. 2 B). As a result, there were no significant differences in NE overflow between control (0.580±0.125ng/g, n=5) and insulin plus cocaine-treated mesenteric arteries (0.543±0.115ng/g, n=5).

Effect of ouabain

5×10^{-4}M ouabain alone increased NE overflow significantly (0.546±0.065 vs 0.407±0.037ng/g, $p<0.01$,n=4). The addition of 1U/l insulin to the ouabain-treated mesenteric preparation failed to decrease NE overflow (0.554±0.088ng/g vs 0.546±0.065ng/g, ns, n=4) (Fig. 3).

Discussion

Our data firstly demonstrate that physiological insulin doses did not change pressor responses to NE bolus injection. Pharmacological doses of insulin have previously been shown to attenuate pressor responses to exogenous NE (4) and to have vasodilating effects(1). In their report, smaller

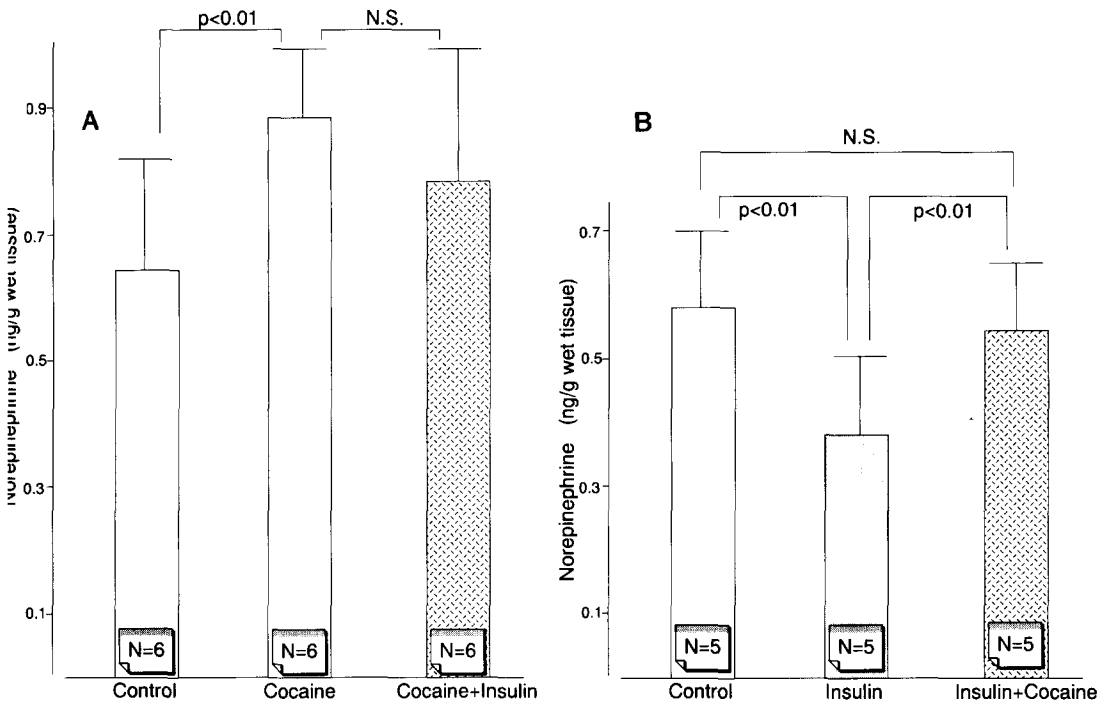

Figure 2. Effect of cocaine on electrically stimulated norepinephrine (NE) release from peripheral sympathetic nerves.
A: Mesenteric arteries were isolated with the accompanying nerve plexi and preincubated in 5×10^{-5} M cocaine (Cocaine) and additional administration of 1U/l insulin (Cocaine+Insulin). NE overflow was assayed as described in Materials and Methods.
Note that total NE overflow was significantly elevated by cocaine. But the addition of 1U/l insulin on cocaine-treated preparation had no effect on total NE overflow.
B: Mesenteric arteries were isolated with the accompanying nerve plexi and preincubated in 1U/l insulin (Insulin) and additional administration of 5×10^{-5} M cocaine (Insulin+Cocaine). NE overflow was assayed as described in Materials and Methods.
Note that insulin decreased NE overflow and the addition of cocaine on insulin-treated preparation reversed total NE overflow to control level.
means±SD.
N stands for the number of rats.

insulin doses have no effect, which is consistent with our data. Pressor responses to NE bolus injection mainly reflects vascular smooth muscle contractility but does not reflect NE reuptake or metabolism. This evidence confirms that physiological insulin doses do not attenuate the contraction of vascular smooth muscle.

We also demonstrate that insulin attenuates NE overflow by nerve stimulation. This change was not due to time-dependent changes of the preparation because NE overflow did not change significantly in at least 10 consecutive electrical stimulations. NE overflow depends on three factors: NE release from nerve endings, inactivation of the amine group, and NE reuptake

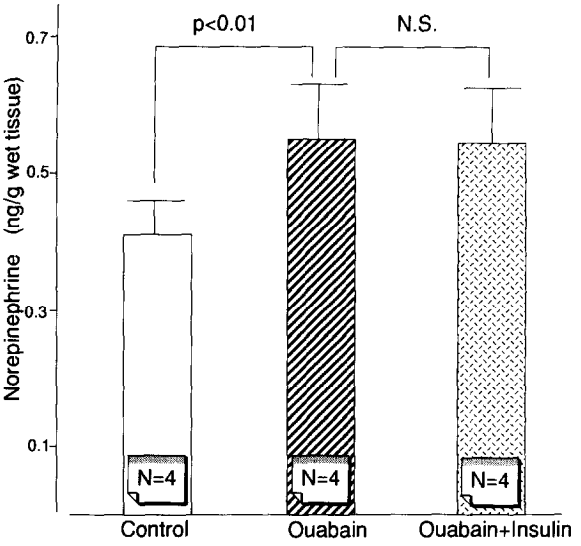

Figure 3. Effect of ouabain on electrically stimulated norepinephrine (NE) overflow from peripheral sympathetic nerves.

Mesenteric arteries were isolated with the accompanying nerve plexi and preincubated in 5×10^{-4}M of ouabain (Ouabain) and additional administration of 1U/l insulin and NE overflow was assayed as described in Materials and Methods.

Note that ouabain increased NE overflow and the addition of insulin had no effect on NE overflow .

means±SD.

N stands for the number of rats.

by nerve terminals. We show that altered NE reuptake may be the main etiology of insulin reduced NE overflow, since pretreatment with cocaine, (a NE uptake blocker), inhibited reduction of NE overflow by insulin (Fig. 2 A). In addition, the addition of cocaine to insulin-treated nerves reversed the effect of insulin (Fig. 2 B). It should be noted that in experiments, insulin was applied only in one dose (1U/l) and the duration of insulin exposure (15 min) was shorter than the experiment of insulin dose dependency (45 min). This difference could explain the relatively weak effects of 1U/l of insulin after exposure of the nerves to cocaine.

It has been shown that neuronal uptake of NE is mediated by a carrier system which is linked to Na^+-K^+-ATPase (5). Insulin facilitates the activity of Na^+-K^+-ATPase (6), Possibly resulting in increased NE reuptake followed by attenuation of NE overflow. This hypothesis is supported by our finding that pretreatment with Na^+-K^+-ATPase inhibitor ouabain completely blocked the effects of insulin on NE overflow (Fig. 3).

It has further been shown that insulin does not affect the activities of enzymes such as monoamine oxidase or catechol-*O*-methyl transferase, which are responsible for NE degradation (7), and inhibition of these enzymes does not increase NE overflow. Therefore it is unlikely that enhanced NE

degradation causes the insulin-induced attenuation of NE overflow. It remains that insulin may simply inhibit NE release from nerve endings. Intracellular calcium is required for NE release from nerve terminals (8), and insulin may decrease intracellular calcium through attenuated Na^+-Ca^{2+} exchange by enhanced Na^+-K^+ -ATPase activity. As a result, insulin could decrease NE overflow partly by inhibiting NE release from nerve terminal. However, the main cause of insulin-induced decrease in NE overflow may be the increased NE uptake, since cocaine completely abolished the effect of insulin.

The attenuation of NE overflow by insulin could explain the acute hypotensive effect of insulin observed in diabetic patients (9). It has been shown that insulin decreases blood pressure, and results in forearm and cutaneous finger vasodilation in the patients. Physiological doses of insulin do not attenuate the basal vascular response to exogenous NE (4), however, it does attenuate NE overflow by nerve stimulation. Therefore, we suggest that enhanced NE reuptake from peripheral sympathetic nerve endings by insulin may cause vasodilatation, leading to the transient hypotension often observed after insulin administration (9).

In conclusion, we have demonstrated that insulin attenuated electrically-stimulated NE overflow, which can be reversed by both treatment with cocaine or ouabain. Therefore, the effect of insulin may be due to increased NE reuptake via activation of the Na^+-K^+- ATPase pump.

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