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# Hepatic insulin sensitizing substance: a novel 'sensocrine' mechanism to increase insulin sensitivity in anaesthetized rats

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**1** We recently described the sensory nitrergic nature of the hepatic insulin sensitizing substance (HISS) mechanism linked to postprandial activation of anterior hepatic plexus fibres in rabbits. This study is designed to assess the involvement of the sensory pathways in this mechanism.

**2** Selective sensory denervation of the anterior hepatic plexus (AHP) was achieved by a 3-day perineurial treatment with 2% capsaicin solution in Wistar rats (230–250 g). After 1 week, hyperinsulinaemic (100 $\mu$U kg$^{-1}$) euglycaemic (5.5 mmol kg$^{-1}$) glucose clamp studies were performed to estimate insulin sensitivity.

**3** The rats with regional AHP sensory denervation exhibited a significantly decreased insulin sensitivity, that is, $9.1 \pm 1.0$ mg kg$^{-1}$ min$^{-1}$ glucose reinstalled euglycaemia vs $13.3 \pm 1.9$ mg kg$^{-1}$ min$^{-1}$ glucose ($P < 0.01$) in control rats.

**4** Acute partial hepatic denervation by AHP cut was without effect on insulin sensitivity, whereas chronic hepatic denervation induced insulin resistance was similar to that achieved by regional AHP capsaicin treatment.

**5** Intraportal administration of L-NAME (10 mg kg$^{-1}$) decreased, whereas capsaicin (0.3 mg kg$^{-1}$ min$^{-1}$) increased insulin sensitivity. Neither atropine (1 mg kg$^{-1}$) nor acetylcholine (1–10 $\mu$g mg min$^{-1}$) produced any significant effect. In animals with preceding regional capsaicin desensitization, none of the pharmacological manoeuvres modified the resulting insulin-resistant state.

**6** Cysteamine (200 mg kg$^{-1}$ s.c.) is known to cause functional somatostatin depletion-induced insulin resistance similar to that produced by either chronic partial hepatic denervation or perineurial AHP capsaicin desensitization. Intraportal capsaicin (0.3 mg kg$^{-1}$ min$^{-1}$) was unable to modify insulin resistance achieved by cysteamine.

7 We conclude that capsaicin-sensitive sensory fibres play a crucial role in neurogenic insulin sensitization known as the HISS mechanism without involvement of anatomical reflex-mediated circuits. The results also suggest that HISS is identical to somatostatin of AHP sensory neural origin. *British Journal of Pharmacology* (2003) **139,** 1171–1179. doi:10.1038/sj.bjp.0705342

**Keywords:** Capsaicin; sensory nerves; denervation; insulin resistance; hyperinsulinaemic euglycaemic glucose clamp; hepatic insulin sensitizing substance; rat

**Abbreviations:** AHP, anterior hepatic plexus; CGRP, calcitonin gene-related peptide; HEGC, hyperinsulinaemic euglycaemic glucose clamp; HISS, hepatic insulin sensitizing substance; L-NAME, *N*(G)-nitro-L-arginine methyl ester; NO, nitric oxide; RIST, rapid insulin sensitivity test; SIN-1, 3-morphonylosydnonimide; SNP, sensory neuropeptide; TRPV1, vanilloid receptor type 1 of the transient receptor potential channel family; VDCC, voltage-dependent $Ca^{2+}$-ion channel; VR1, vanilloid receptor type 1

## Introduction

Postprandial activation of nerve fibres in the anterior hepatic plexus leads to the release of a hormone-like acting substance termed HISS (hepatic insulin sensitizing substance), which increases the sensitivity of peripheral tissues to the hypoglycaemic effect of insulin (Sadri & Lautt, 1999). A series of experiments published by Lautt's group suggests that the release of HISS is a consequence of a parasympathetic reflex activation, a process anatomically linked to the anterior hepatic plexus, which is initiated by postprandial hyperinsulinaemia (Xie & Lautt, 1995; Xie & Lautt, 1996b; Sadri & Lautt, 1999). The mechanism is significantly influenced by hepatic nitric oxide (NO) production, since intraportal, but not systemic administration of a relatively low dose of *N*-nitro-L-arginine methyl ester (L-NAME), a nonselective NO synthase inhibitor, caused significant insulin resistance (Sadri & Lautt, 1999). Alternatively, intraportal, but not intravenous administration of 3 morphonylosydnonimide (SIN-1), a nonenzymatic NO donor partially restored insulin sensitivity in

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animals made insulin resistant by preceding application of *N*-monomethyl-L-arginine, another nonselective NO synthase inhibitor (Sadri & Lautt, 1999).

We have found that activation of nitrergic nerves, a significant part of which belongs to capsaicin-sensitive primary afferents, is involved in several cardiovascular adaptive mechanisms such as the ischaemic preconditioning phenomenon (Ferdinandy *et al*., 1997) and the regulation of coronary tone (Oroszi *et al*., 1999a,b). In a very recent study, we found that the HISS mechanism was completely abolished when sensory nerves in the AHP were functionally blocked by means of a preceding 3-day exposure to 2% perineurial capsaicin solution in rabbits (Porszasz *et al*., 2002). The AHP contains a significant amount of capsaicin-sensitive sensory nerve fibres (Miao *et al*., 1997; Erin *et al*., 2000). It has also been shown that activation of capsaicin-sensitive fibres results in the release of both sensory neuropeptides and acetylcholine derived from intrinsic neurons and parasympathetic efferents in the gastrointestinal tract (Bartho & Holzer, 1995; Weber *et al*., 2001) serving as a cholinergic–nitrergic efferent pathway to regulate gastrointestinal motility and secretion. An aim of the present work was therefore to elucidate whether the HISS mechanism resulted from a cholinergic–nitrergic reflex activation or it may reflect the sensory-effector function of AHP fibres. The latter is known to occur through neuropeptide release from activated sensory nerve terminals without any involvement of reflex-mediated circuits (Szolcsányi, 1996; Németh *et al*., 2003). As a major methodological approach, we used hyperinsulinaemic euglycaemic glucose clamping, the gold standard to estimate insulin sensitivity *in vivo* (DeFronzo *et al*., 1979). To confirm the involvement of AHP sensory fibres in the HISS mechanism (Porszasz *et al*., 2002), these fibres were subjected to a 3-day perineurial capsaicin exposure, an intervention known to induce a long-lasting sensory neuropeptide deficiency with a loss of sensory effector function of the exposed fibres (Szolcsanyi *et al*., 1998a,b). To attain an immediate and selective activation of AHP sensory fibres, intraportal capsaicin infusion was applied. Similarly, substances with a suspected modulatory effect on the HISS mechanism, such as acetylcholine or NO synthase inhibitors (Lautt, 2003) were administered into the portal vein. Acute and chronic partial hepatic denervation was applied by complete AHP cut to assess contribution of AHP-linked reflex-mediated pathways to this endogenous insulin sensitizing mechanism. The results obtained suggest that the HISS mechanism is executed by capsaicin-sensitive AHP fibres without significant involvement of cholinergic reflex-mediated pathways in anaesthetized rats. Moreover, it also seems likely that HISS is identical to somatostatin of sensory neural origin.

## Methods

### *Ethics*

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical boards of Medical Universities of Pecs and Debrecen, Hungary (license No:42/2001 DEMAB).

### *Experimental animals*

Adult male Wistar rats weighing 230–250 g, housed in an animal room (12-h light/dark periods a day, temperature of 22–25°C, humidity of 50–70%) with two animals per pen, fed commercial laboratory chow and tap water *ad libitum*. The animals underwent the experimental procedures after a 2-week adaptation period. Each animal was subjected to hyperinsulinaemic euglycaemic clamp studies after a 24-h period of fasting with free access to water.

### *Animal preparation for plexus hepaticus anterior stimulation studies*

*Surgery* The animals were anaesthetized with an initial intraperitoneal dose of 70 mg kg$^{-1}$ of thiopental sodium. Continuous anaesthesia was maintained by succeeding intravenous infusion of thiopental sodium solution of 1.5 mg kg$^{-1}$ 100 g$^{-1}$ body weight through a cannula inserted into the right femoral vein. Assisted artificial respiration was applied when necessary to maintain arterial $pCO_2$ below 40 mmHg as described previously (Szilvassy *et al*., 1994.) (This type of anaesthesia was applied at each experimental protocol used in the present study). The abdominal cavity was opened, the AHP was prepared, cleaned of fat and adhering connective tissues. Since nerve fibres in the posterior hepatic plexus have been excluded from involvement in the HISS mechanism (Xie & Lautt, 1995; Xie & Lautt, 1996a; Sadri & Lautt, 1999), our study protocols included various experimental manipulations on the anterior plexus, that is, electrical stimulation, surgical or chemical denervation. The plexus was cut as high as possible and strains of 50 Hz double threshold square-wave stimuli (500 is) were then applied to bundles distal to the site of nerve cut through pairs of platinum electrodes. For nerve stimulation studies, both the stimulating electrodes and the corresponding nerve bundles were immersed in liquid paraffin to avoid physical nerve damage (Szolcsanyi, 1996; Szolcsanyi *et al*., 1998a,b).

For hyperinsulinaemic euglycaemic glucose clamp studies, two venous catheters were placed in the two external jugular veins for insulin and glucose infusion. In addition, an arterial cannula was inserted into the right carotid artery for arterial blood glucose measurement.

*Perineurial capsaicin desensitization* Bundles of AHP were prepared, cleaned of fat and adhering connective tissues. Surgical sponge slices of approximately 3 mm length impregnated with 2% capsaicin solution were applied around the plexus. The abdominal cavity was closed and the perineurial sponge slices were left in their places over 3 days. The abdomen was then reopened and the sponge pieces were removed. The wounds were closed and a week was allowed for each animal to recover. After the period of convalescence, the animals were subjected to hyperinsulinaemic euglycaemic clamp studies. For control to this series of rats served those which received matching sponge pieces impregnated with the solvent for capsaicin (ethanol + Tween 80).

### *Hyperinsulinaemic euglycaemic glucose clamp studies*

Human regular insulin (NOVO Nordisk, Copenhagen) was infused at constant rates of 8.0. and 13.0 mU kg$^{-1}$ min$^{-1}$ *via*

one of the jugular venous catheters over 120 min to attain stable plasma insulin immunoreactivity of $50 \pm 5$ and $100 \pm 5\ \mu U\ ml^{-1}$, respectively, in the steady state (see below). Blood samples (0.2 ml) were taken from the arterial cannula for blood glucose concentration measurement at 10 min intervals. Blood glucose concentration was maintained constant ($5.5 \pm 0.5\ mmol\ l^{-1}$) by a variable rate of 20% glucose infusion *via* the second jugular venous cannula. When blood glucose had stabilized for at least 30 min, we defined this condition as steady state. In the steady state, additional blood samples (0.3 ml) were taken for plasma insulin determination three times at 10-min intervals. The glucose infusion rate ($mg\ kg^{-1}\ min^{-1}$) during steady state was used to characterize insulin sensitivity (DeFronzo *et al*., 1979; Porszasz *et al*., 2002).

### *Drug study design*

Figure 1 shows the design of the study. In each group, insulin sensitivity determined by hyperinsulinaemic euglycaemic glucose clamping served as end point. The first series of experiments was to confirm the involvement of capsaicin-sensitive AHP sensory fibres in the HISS mechanism in anaesthetized rats, similar to that found in conscious rabbits (Porszasz *et al*., 2002). This was achieved by selective deterioration of AHP sensory fibres by means of perineurial capsaicin treatment (group 1) as described above. As control to this series of rats those which received matching placebo sponge slices (group 2) served. To elucidate whether AHP-linked reflex-mediated pathways were involved in the HISS mechanism, we determined insulin sensitivity after acute AHP nerve cut (group 3) using sham-operated control animals (group 4). In a separate group of rats, chronic partial hepatic denervation was accomplished by AHP cut 1 week preceding determination of insulin sensitivity (group 5) using sham-operated controls (group 6). This latter protocol served as another approach to study the effect of selective degeneration of AHP fibres on tissue insulin sensitivity. Since the deterioration of AHP sensory fibres yielded insulin resistance (Porszasz *et al*., 2002), we assumed that either electrical (group 7) or chemical stimulation of these fibres by 0.3 mg intraportal capsaicin over 5 min (group 8) would produce an increase in insulin sensitivity. In separate sets of experiments, we intended to confirm the sensitivity of the HISS mechanism to NO synthase inhibition (animals in group 9 were given $10\ mg\ kg^{-1}$ intraportal L-NAME as a bolus) and atropine (group 10 animals were given $1\ mg\ kg^{-1}$ intraportal atropine) as well as to reproduce the results by Lautt *et al*. (2001), that is, to try to activate the mechanism by intraportal acetylcholine infusion ($1-10\ \mu g\ kg^{-1}\ min^{-1}$, group 11) using euglycaemic clamping as end point. This latter series of experiments was carried out both with (12–15) and without (groups 8–11) preceding perineurial AHP capsaicin desensitization. Since from the above experiments we obtained evidence for the sensory nitrergic nature of the HISS mechanism without involvement of reflex-mediated pathways (see results) in anaesthetized rats, we concluded that this endogenous insulin sensitizing process appeared as a sensocrine (Szolcsanyi *et al*., 1998a,b) mechanism linked to AHP sensory fibres. It was therefore tempting to assume that HISS were a sensory neuropeptide entering the circulation and acting as an endogenous insulin sensitizer. Since among the sensory neuropeptides somatostatin is known to increase insulin sensitivity (Bruttomesso *et al*., 2001), and it has been described as a sensocrine mediator, rats in a distinct group were devoted to study insulin sensitivity after a preceding $200\ mg\ kg^{-1}$ s.c. cysteamine administration shown to cause functional somatostatin depletion (Szolcsanyi *et al*., 1998a,b).

### *Statistical analysis*

The results are expressed as means ± standard deviation (s.d.) obtained with six animals per group. The data were analysed with repeated-measures ANOVA followed by Student's *t*-test modified according to Bonferroni's method. Changes were considered statistically significant at $P < 0.05$.

## Results

### *Effect of regional capsaicin desensitization on insulin sensitivity*

Regional capsaicin desensitization, executed by wrapping AHP nerve branches into surgical sponge envelopes impregnated with 2% capsaicin solution, yielded a significant decrease in insulin sensitivity as determined by hyperinsulinaemic ($100\ \mu U\ ml^{-1}$) euglycaemic ($5.5\ mmol\ l^{-1}$) clamp studies 1 week after sponge removal. The control animals in either group disclosed similar insulin sensitivity values (Figure 2).

### *Effect of intraportal L-NAME, capsaicin, atropine and acetylcholine on insulin sensitivity with and without preceding regional capsaicin desensitization*

Intraportal administration of L-NAME ($10\ mg\ kg^{-1}$) decreased, whereas capsaicin ($0.3\ mg\ kg^{-1}\ min^{-1}$) increased insulin sensitivity. Neither atropine ($1\ mg\ kg^{-1}$ intraportally) nor acetylcholine ($1-10\ \mu g\ kg^{-1}\ min^{-1}$ intraportally) produced any significant effect. In animals with preceding regional capsaicin desensitization, none of the pharmacological manoeuvres modified the resulting insulin resistant state (Figure 3).

### *Effect of electrical nerve stimulation on insulin sensitivity at various degrees of clamped hyperinsulinaemia subsequent to partial acute hepatic denervation*

It is shown in Figure 4 that nerve stimulation failed to modify insulin sensitivity when plasma insulin immunoreactivity was clamped at $100\ \mu U\ ml^{-1}$. However, when plasma insulin was clamped at $50\ \mu U\ ml^{-1}$, nerve stimulation attained a significant increase in insulin sensitivity as compared to control (i.e. in the absence of stimulation after nerve cut). Interestingly, acute nerve cut did not elicit any change in insulin sensitivity by itself (see control values in Figures 3 and 4). The same nerve stimulation protocol did not modify the decreased insulin sensitivity in animals with preceding perineurial treatment with capsaicin, irrespective of the degree of hyperinsulinaemia.

### *Different effects of acute vs chronic partial hepatic denervation on insulin sensitivity*

Acute denervation (transversal cut of AHP fibres) did not modify insulin sensitivity. However, the

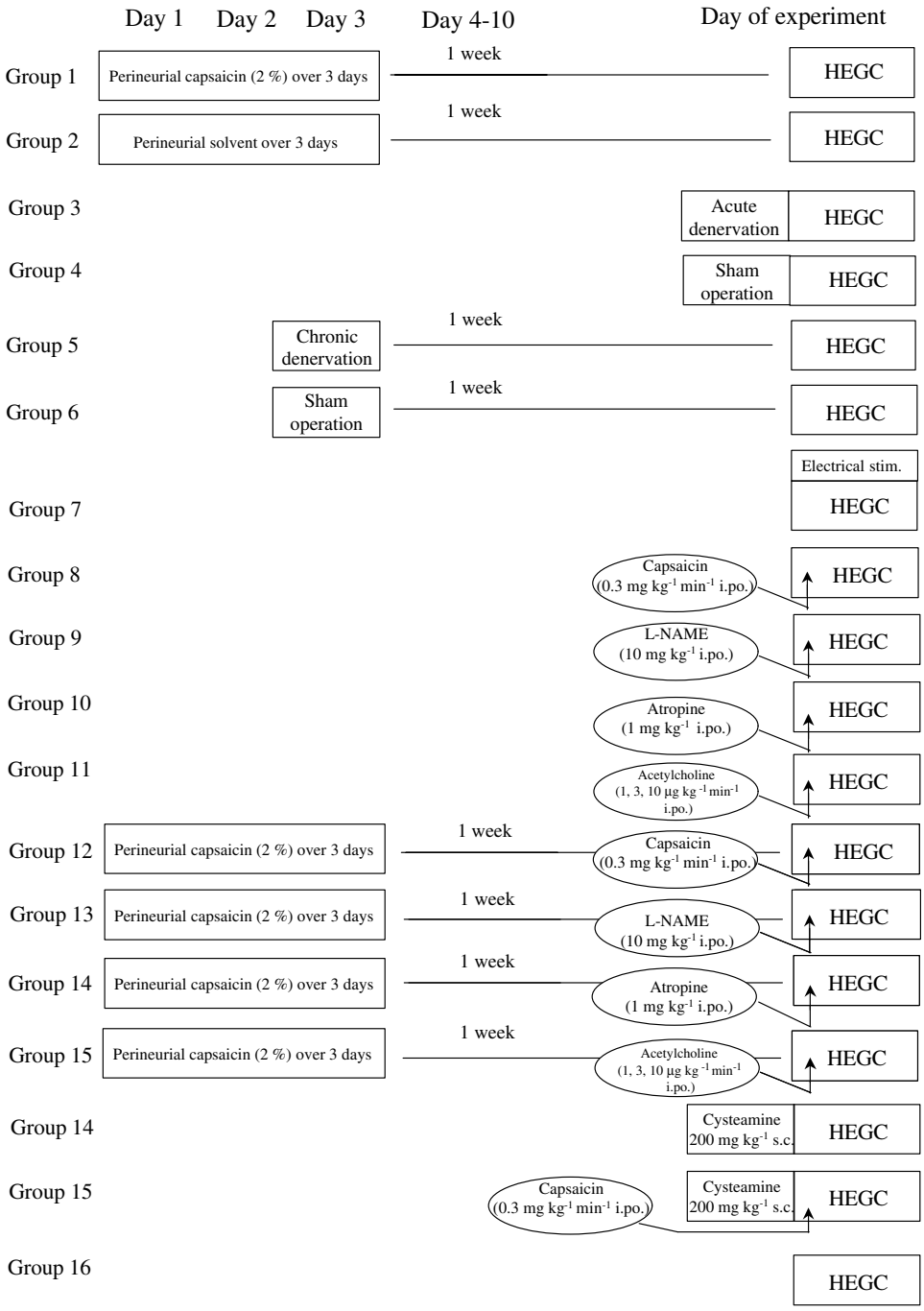


**Figure 1** Schematic drawing of the study design. In each group, insulin sensitivity was determined by means of hyperinsulinaemic euglycaemic glucose clamp (HEGC). The AHP of animals in group 1 was wrapped in Gelaspon sponge pieces impregnated with 2% capsaicin solution. In case of group 3, acute denervation was applied at the beginning of the experiment. Chronic partial hepatic denervation (cross-section of the anterior hepatic plexus) was performed in animals belonging to group 5, and the experiments were commenced a week succeeding denervation. The groups with even numbers (groups 2, 4, 6) represent the corresponding control groups. In the animals of group 7, electrical stimulation (30 V; 0.5 ms; 50 Hz; 10 min) of AHP was performed. Capsaicin was infused ($0.3\,mg\,kg^{-1}\,min^{-1}$) into the portal vein of animals belonging to group 8. In groups 9, 10 and 11, L-NAME ($10\,mg\,kg^{-1}\,min^{-1}$), atropine ($1\,mg\,kg^{-1}$) and acetylcholine (1, 3 or $10\,\mu g\,kg^{-1}\,min^{-1}$) were infused. The latter series of experiments (groups 8–11) were carried out with preceding perineurial AHP capsaicin desensitization (groups 12–15) as well. The animals of groups 14 and 15 were treated by $200\,mg\,kg^{-1}$ cysteamine 30 min before the commencement of the experiment and capsaicin ($0.3\,mg\,kg^{-1}\,min^{-1}$) was infused in group 14. Clamping was performed without treatment as a control experiment (group 16).

hyperinsulinaemic euglycaemic clamp studies, carried out 1 week after surgical denervation, revealed a significantly decreased insulin sensitivity. Intraportal capsaicin infusion significantly increased insulin sensitivity after acute but not chronic partial hepatic denervation (Figure 5).

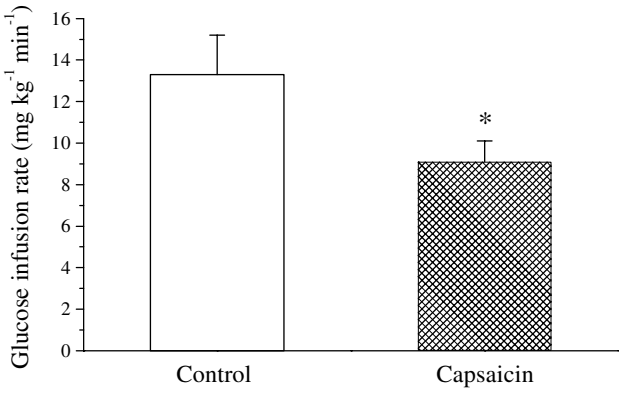


**Figure 2** Effects of regional capsaicin desensitization on insulin sensitivity. Regional desensitization was induced by wrapping the AHP fibres up with Gelaspon sponges impregnated with 2% capsaicin solution. The data are expressed as means ± s.d. obtained with six animals per group.*$P<0.05$.

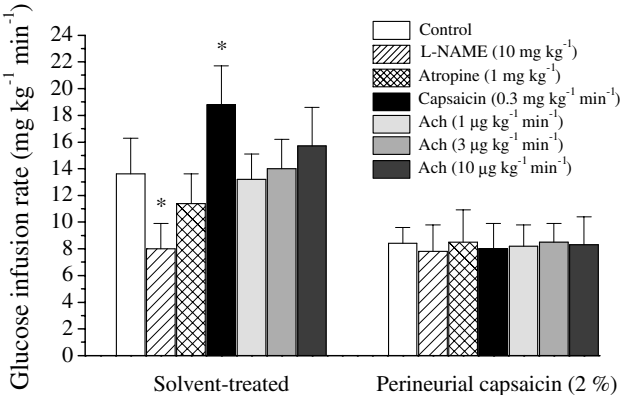


**Figure 3** Effects of perineurial treatment of the AHP with capsaicin/its solvent on insulin sensitivity in anaesthetized rats and the modifying effects of L-NAME (hatched column), atropine (crosshatched column), or infusion of capsaicin (black column), acetylcholine (grey columns) into the portal vein. Capsaicin was applied as perineurial sponges impregnated with 2% capsaicin solution. The data are expressed as means ± s.d. obtained with six animals per group.*$P<0.05$.

### *Effect of intraportal* L-*NAME on intraportal capsaicin-induced increase in insulin sensitivity. A reversal by* L-*arginine*

In this set of experiments, we studied the NO dependency of the insulin-sensitizing effect of intraportal capsaicin, as a specific activator of AHP sensory fibres. As shown from results in 'Figure 6', intraportal capsaicin infusion produced a dose-dependent increase in insulin sensitivity, whereas intraportal L-NAME ($10\,mg\,kg^{-1}$) induced insulin resistance. Capsaicin was able to increase insulin sensitivity in the presence of NO synthase inhibition; however, this effect was much weaker than in the absence of L-NAME. The insulin sensitivity decreasing effect of L-NAME was completely reversed by intraportal L-arginine ($100\,mg\,kg^{-1}$) either with or without capsaicin.

### *Cysteamine-induced insulin resistance*

Cysteamine ($200\,mg\,kg^{-1}$ s.c.), a selective somatostatin-depleting agent, induced a significant decrease in tissue insulin

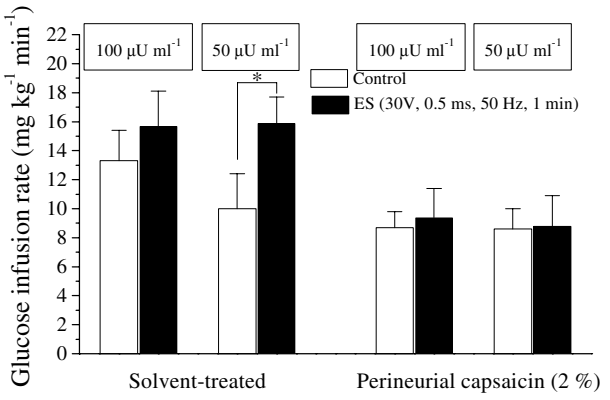


**Figure 4** Effects of perineurial capsaicin treatment (2%) of AHP on glucose infusion rate to maintain euglycaemia ($5.5\,mmol\,l^{-1}$) at selected degrees of clamped hyperinsulinaemia (100 and $50\,iU\,ml^{-1}$) with or without electrical stimulation (ES) of AHP. The data are expressed as means ± s.d. obtained with six animals per group. *$P<0.05$.

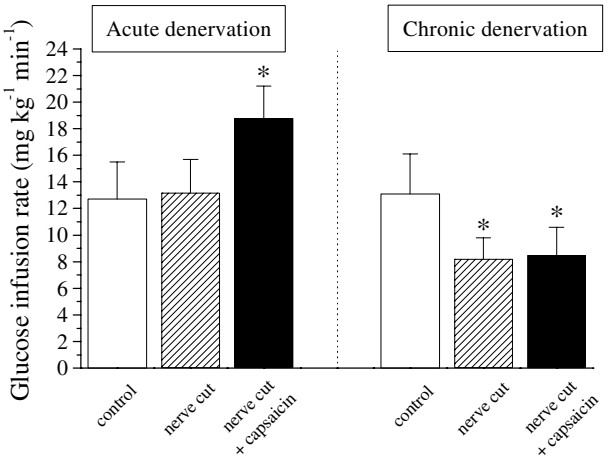


**Figure 5** The effect of acute vs chronic partial hepatic denervation on insulin sensitivity determined by hyperinsulinaemic euglycaemic glucose clamping in anaesthetized rats. Chronic denervation means that the glucose clamp study was carried out 1 week after surgical denervation. Acute denervation means that the glucose clamp study was carried out immediately after nerve cut. Capsaicin was infused into the portal vein at a dose of $0.3\,mg\,kg^{-1}\,min^{-1}$ over 5 min in both acute and chronic denervation states. The data are expressed as means ± s.d. obtained with 6 animals per group. *$P<0.05$.

sensitivity applied 30 min before commencement of glucose clamping. Moreover, this manoeuvre completely abolished the insulin sensitizing effect of intraportal capsaicin (Figure 7)

## Discussion

This work in part is an extension of our previous results obtained in rabbits that demonstrated functional deterioration of sensory nerve fibres in the AHP yields the development of insulin resistance or acute stimulation of these fibres by intraportal capsaicin increases insulin sensitivity (Porszasz *et al.*, 2002). Since according to the present results, blockade of NO synthesis by L-NAME significantly attenuated the HISS effect, the sensory nitrergic nature of the HISS mechanism has now been confirmed in two species, that is, in rabbits (Porszasz

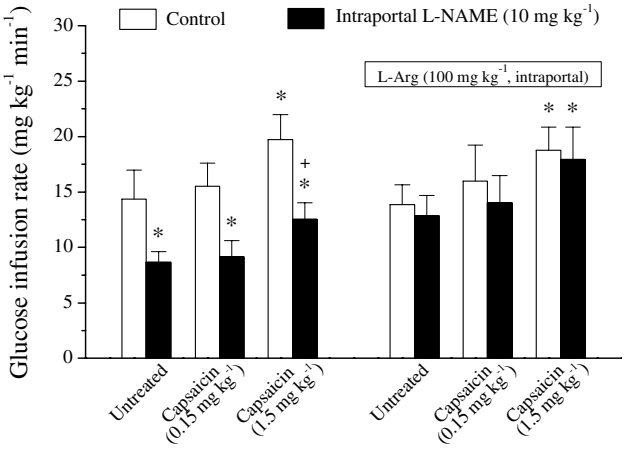


**Figure 6** Effect of intraportal L-NAME on intraportal capsaicin-induced increase in insulin sensitivity and the reversal of L-arginine in the anaesthetized rat. The glucose infusion rate ($mg\,kg^{-1}\,min^{-1}$) was measured after intraportal L-NAME ($10\,mg\,kg^{-1}$) (filled bars) administration comparing with the corresponding controls (open bars) with or without intraportal L-arginine ($100\,mg\,kg^{-1}$) injection. Intraportal capsaicin induced a dose-dependent increase of insulin sensitivity independently of L-arginine administration. L-NAME-induced insulin resistance, which was completely reversed by L-arginine. The L-NAME induced deterioration of insulin sensitivity was ameliorated by capsaicin. The data are expressed as means ± s.d. obtained with 6 animals per group. $^{*}P<0.05$ compared to the corresponding control; $^{+}P<0.05$ compared to the effect of the low dose ($0.15\,mg\,kg^{-1}$) of capsaicin.

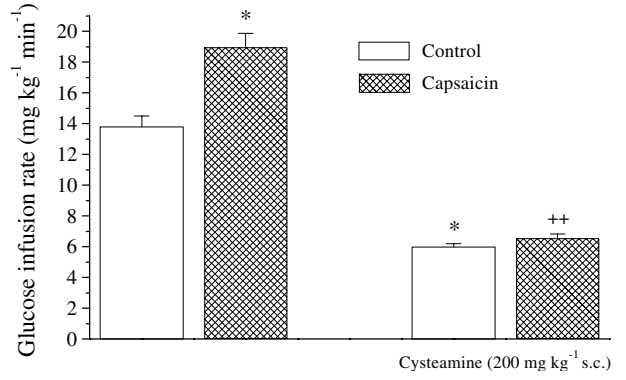


**Figure 7** Effect of cysteamine ($200\,mg\,kg^{-1}$ s.c.) on insulin sensitivity. The subcutaneously injected cysteamine induced a significant decrease in insulin sensitivity applied 30 min before the commencement of glucose clamping. This somatostatin-depleting agent was able to prevent and reverse the development of insulin sensitivity increase induced by intraportal capsaicin ($0.3\,mg\,kg^{-1}\,min^{-1}$) administration. The data are expressed as means ± s.d. obtained with eight animals per group. $^{*}P<0.05$ compared to the control without cysteamine administration; $^{++}P<0.01$ compared to the effect of capsaicin without cysteamine administration.

*et al*., 2002) and rats. However, the original findings of the present work are that activation of this endogenous insulin sensitizing mechanism does not involve anatomical reflex-mediated circuits. This has been evidenced by that acute cut of the AHP fibres was without influence on insulin sensitivity. On the contrary, chronic partial hepatic denervation by AHP cut 1 week preceding determination of insulin sensitivity developed a marked insulin resistant state. This result taken together with the sensitivity of the HISS mechanism to regional capsaicin desensitization which is known to induce a transient neuropeptide loss, strongly suggests the role of sensory neuropeptides in inducing or mediating the HISS effect. Indeed, the HISS effect was lost after administration of $200\,mg\,kg^{-1}$ cysteamine, a somatostatin-depleting agent Szolcsanyi *et al*., 1998a, suggesting that HISS were identical to somatostatin of AHP sensory neural origin.

Capsaicin has been described to attain an effect exclusively on sensory nerves possessing vanilloid receptors (Caterina *et al*., 1997). Therefore, our results strongly confirm the involvement of sensory fibres in neurogenic regulation of insulin sensitivity in rats as well. Pretreatment of adult rats with high doses of capsaicin is known to produce chronic neuropeptide depletion, which in turn results in deterioration of the sensory effector function of capsaicin-sensitive nerves (Jancso *et al*., 1977; Szolcsanyi, 1996). However, an acute exposure to capsaicin leads to a nonselective cation channel activation, which leads to the release of sensory neuropeptides such as calcitonin gene-related peptide (CGRP), substance P or somatostatin (Jancso *et al*., 1977; Szolcsanyi, 1996; Szolcsanyi *et al*., 1998a,b). Thus, the decrease in insulin sensitivity as a consequence of regional capsaicin desensitization is possibly based on the loss of the effector function of capsaicin-sensitive sensory nerves in the anterior hepatic plexus. Similarly, an increase in insulin sensitivity in response to intraportal administration of capsaicin is a consequence of intrahepatic sensory nerve activation.

That nerves in the AHP play a crucial role in the regulation of postprandial increase in insulin sensitivity in rats was first described in the pioneering works of Lautt's group (Xie & Lautt, 1995; Xie & Lautt, 1996a; Sadri & Lautt, 1999). This discovery was then principally confirmed by Moore *et al*. (2002), who found that chronic partial hepatic denervation induced insulin resistance in dogs. However, the latter group carried out insulin sensitivity tests 16 days after hepatic denervation, whereas Lautt *et al*. (2001) found acute hepatic denervation to produce insulin resistance in rats. According to the original concept of Lautt's group, the insulin sensitizing mechanism linked to functional integrity of the AHP fibres worked as follows: an insulin sensitizing parasympathetic reflex was activated in the liver in response to postprandial hyperinsulinaemia, the atypical efferent pathway of which involves the release of HISS, an unidentified hormone-like substance that increases the sensitivity of peripheral tissue (predominantly the skeletal muscle) to the hypoglycaemic effect of insulin (Xie *et al*., 1993; Xie & Lautt, 1994, 1996a; Sadri *et al*., 1997; Lautt *et al*., 2001). This mechanism was shown to involve some nitrergic components, since various NO synthase inhibitors apparently induced insulin resistance, and intraportal administration of NO donors restored physiological insulin sensitivity in such animals (Sadri & Lautt, 1999). Our results seem to support only partially this assumption. As shown from results in Figures 4 and 5, acute nerve cut of the AHP fibres preceding the nerve stimulation studies did not modify insulin sensitivity by itself. Moreover, neither atropine nor intraportal acetylcholine affected insulin sensitivity at least under our experimental conditions. Theoretically, the discrepancy may in part be explained by a difference in the way of determining insulin sensitivity. Lautt's group used the rapid insulin sensitivity test termed RIST (Lautt *et al*., 1998) to characterize changes in insulin sensitivity, whereas we remained with using the hyperinsulinaemic euglycaemic

glucose clamping, the gold standard of determining insulin sensitivity in whole animals (DeFronzo *et al*., 1979). The RIST method utilizes hyperinsulinaemia produced by a short-term insulin infusion (usually 5 min), the hypoglycaemic effect of which is compensated by a succeeding longer lasting glucose infusion (Lautt *et al*., 1998). During the hyperinsulinaemic clamp method, however, plasma insulin immunoreactivity is maintained at a level approximately 10 times higher ($100\,\mu U\,ml^{-1}$) than corresponding fasting values with confirmation by succeeding radioimmunoassay determinations and accompanied by continuous glucose infusion. Assuming that the HISS mechanism is activated by hyperinsulinaemia, it is possible that a much higher degree of the HISS mechanism activation is achieved by clamping (because of the long-lasting stable hyperinsulinaemia) than by the RIST method. This may serve as an explanation for the lack of effect of either intraportal acetylcholine infusion or electrical stimulation of peripheral anterior hepatic plexus fibres subsequent to an acute nerve cut to further increase insulin sensitivity. This is further suggested by the increase in insulin sensitivity attained by the same nerve stimulation protocol at lower ($50\,\mu U\,ml^{-1}$) clamped plasma insulin level. Based on the comparative study by Reid *et al*. (2002), it is also suggested that the HISS mechanism is less detectable with euglycaemic clamping than the RIST method including the effect of atropine. However, that NO synthase inhibition resulted in insulin resistance in our studies as well, and that activation of hepatic sensory fibres by means of intraportal capsaicin infusion even after acute denervation can increase insulin sensitivity, emphasizes the role of sensory nitrergic pathways in this neurogenic insulin sensitizing mechanism. This is in accordance with the observation by Porszasz *et al*. (2002) that intraportal nitroglycerin infusion increased insulin sensitivity in conscious rabbits and that NO donors may improve insulin sensitivity in men (Kovacs *et al*., 2000). Moreover, these results also suggest that the HISS mechanism can be investigated using the hyperinsulinaemic euglycaemic clamp method. We think that this latter statement is not in a significant controversy with that of Lautt's group, who described that the RIST method is more appropriate to study HISS effects than the clamp method (Lautt, 2003), as RIST is essentially a rapidly sampled euglycaemic clamp in response to a pulse of insulin as defined by Lautt himself (2003). Moreover, regardless of the endpoint used for determination of insulin sensitivity, we could demonstrate an insulin-resistant state after partial hepatic denervation and a restoration of insulin responsiveness by intraportal administration of NO donors (Porszasz *et al*., 2002) or capsaicin. Thus, we think that relevance of the clamp method even in context of the HISS mechanism is not questionable. Similarly, Moore *et al*. (2002) were successful in verifying the HISS mechanism in dogs by means of hyperinsulinaemic euglycaemic clamping. As far as the controversy in connection with the modulatory effect of atropine is concerned, we consider possible that the well known and clinically utilizable secondary sensory neuropeptide release inhibitory (antinociceptive) effect of atropine may be more striking with RIST than with clamping (Guimaraes *et al*., 2000; Migliore *et al*., 2002). However, we cannot exclude the possibility that in our study the virtual absence of significant involvement of cholinergic reflex-mediated pathways could result from the relatively long fasting period (24 h), as shown by Lautt (1999).

To the best of our knowledge, this report is the first to explain that capsaicin-sensitive sensory nerve fibres in the hepatic neural network are of major influence on insulin sensitivity without the involvement of autonomic reflex-mediated pathways. This is strongly suggested by the decrease in insulin sensitivity in response to partial sensory denervation of the liver by means of regional capsaicin desensitization of fibres in the anterior plexus, or the increase in insulin sensitivity attained by selective stimulation of these nerves by intraportal capsaicin or electrical nerve stimulation at $50\,\mu U\,ml^{-1}$ plasma insulin values. As far as the mechanism of the involvement of these fibres in activation of the insulin sensitizing mechanism is concerned, it is unlikely that this is a reflex-mediated one, since acute nerve cut was without effect on insulin sensitivity. Chronic selective denervation, however, did induce insulin resistance. Starting from the principle that the effector function of sensory nerves is based on the release of sensory neuropeptides (Holzer, 1992; Brain, 1996) without involvement of reflex-mediated pathways but due to the capsaicin receptor activation itself (Szolcsanyi, 1996; Szolcsanyi *et al*., 1998a,b; Németh *et al*., 2003), we considered possible that HISS was identical to a sensory neurotransmitter with hormone-like activity (Figure 7). As shown by the results from Figure 6, this neuropeptide may be identical to somatostatin, as cysteamine was able to block the HISS mechanism. Moreover, the animals pretreated with $200\,mg\,kg^{-1}$ cysteamine s.c., an intervention known to achieve selective functional somatostatin depletion (Ahren *et al*., 1989; Szolcsanyi *et al*., 1998a,b) revealed insulin resistance similar to that achieved either by perineurial capsaicin desensitization of the AHP fibres, chronic partial hepatic denervation, or NO synthase inhibition. Results by Szolcsanyi *et al*. (1998a,b) provided evidence for such an example, in terms of somatostatin of sensory neural origin serving as a mediator of a systemic anti-inflammatory effect subsequent to antidromic low-frequency sensory nerve stimulation (Szolcsanyi *et al*., 1998a). In their experiments, pretreatment with either cysteamine or polyclonal somatostatin antiserum completely blocked this sensory nerve stimulation-induced anti-inflammatory effect (Szolcsanyi *et al*., 1998a). Therefore, choosing cysteamine pretreatment as an approach to study the relevance of a somatostatin-mediated process in the HISS mechanism, applying the substance 30 min preceding the clamp experiments (1.5–2.5 h prior to reaching the steady state) seemed to be an appropriate option. Cysteamine, at least around the dose applied, has been shown to selectively eliminate somatostatin-like immunoreactivity and somatostatin effects very rapidly subsequent to its subcutaneous administration through forming mixed sulphhydryl groups resulting in a cysteaminyl–somatostatin complex (Lorenson & Jacobs, 1984; Patel & Pierzchala, 1985). As far as the possible involvement of other sensory neuropeptides such as substance P and CGRP in the HISS mechanism is concerned, their role as endogenous insulin sensitizers can nearly be excluded. CGRP is a potent insulin antagonist (Kreutter *et al*., 1993; Koopmans *et al*., 1998), and substance P is without effect on insulin sensitivity.

In summary, the present results shed light on a newly recognized feature of the effector function of sensory nerves. This neurogenic insulin sensitizing mechanism does not seem to be based on a reflex, and parasympathetic components also do not seem to play a major role. However, it is highly sensitive to NO synthase inhibition, which, especially in the

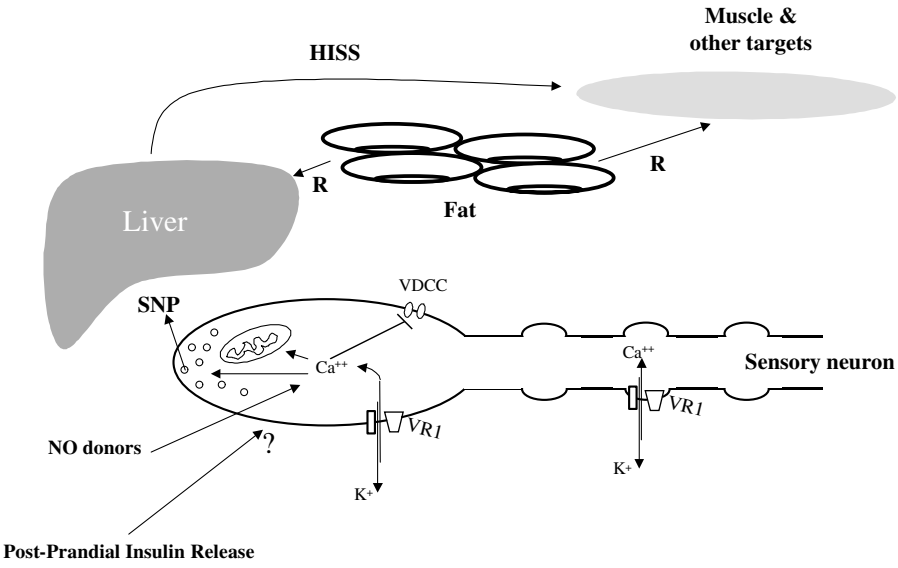


**Figure 8** Schematic representation of the mechanism underlying the release of HISS. Activation of sensory neurons in the AHP in response to either capsaicin or NO donors leads to the release of HISS. Since the activation of sensory neurons is known to result in sensory neuropeptide (SNP) release, it is speculated that HISS is identical to or is released by one of these neuropeptides (somatostatin) from the liver. VDCC: voltage-dependent $Ca^{++}$ ion channels. VR1: capsaicin (TRPV1 vanilloid) receptor. R: resistin.

context of our previous clinical results (Kovacs *et al*., 2000) may deserve some innovative aspects for NO donors as insulin sensitizers or activators of sensory fibres. Thus, in our interpretation, HISS released from AHP sensory fibres in response to either postprandial hyperinsulinaemia or chemicals (nitrates, capsaicin) is possibly identical to somatostatin as a neuropeptide that enters the circulation and sensitizes peripheral tissues to the hypoglycaemic effect of insulin (Figure 8).

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