Effect of capsaicin-sensitive sensory nerves on plasma glucose and catecholamine levels during 2-deoxyglucose-induced stress in conscious rats

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1 Sensory fibres innervate the adrenal medulla but their function is not known. In this paper we have studied the effect of capsaicin-sensitive sensory fibres on the adrenal catecholamine (CA) response and blood glucose response to 2-deoxyglucose (2-DG)-induced glucopenic stress in conscious rats.

2 2-DG at 500 mg kg⁻¹ (i.v.) induced a 2.5 fold increase in plasma glucose levels, a 3.5 fold increase in inferior vena caval (i.v.c.) plasma noradrenaline (NA) levels and a 7 fold increase in i.v.c. plasma adrenaline (Ad) levels over 60 min. The hyperglycaemia in response to 2-DG was attenuated by pentolinium and by left splanchnicotomy plus right adrenalectomy. These procedures also caused a complete inhibition of the increase in plasma CA.

3 The hyperglycaemia in response to 2-DG was attenuated by pretreatment of rats with capsaicin as neonates, suggesting that capsaicin-sensitive sensory fibres are required for regulation of plasma glucose in response to glucopenic stress.

4 The increase in i.v.c. plasma CA levels in response to 2-DG during the early phase of glucopenia (first 30 min) in the conscious rats pretreated with capsaicin was the same as in the rats pretreated with vehicle alone. During the later phase of glucopenia (after 45 min), the increase in plasma CA levels in rats pretreated with capsaicin was higher than in the rats pretreated with vehicle alone.

5 In vehicle-pretreated rats and capsaicin-pretreated rats the tissue NA and Ad levels in the adrenal medulla after 8 h of stress were depleted to the same extent. However, tissue CA levels in the capsaicin group recovered faster over 24 h than in the vehicle group.

6 These results indicate that capsaicin-sensitive sensory fibres are not required to maintain adrenal CA secretion during glucopenic stress in the conscious rat but are required for maintenance of blood glucose levels.

Introduction

Sensory neurones in the dorsal root ganglia and nodose ganglia innervate the adrenal medulla via the splanchnic nerve and vagal nerve (Mohamed *et al.*, 1988; Coupland *et al.*, 1989). Substance P (SP) is present in sensory nerves including those in the splanchnic nerve that innervates the adrenal medulla (Linnoila *et al.*, 1980; Gamse *et al.*, 1981; Pfister & Gorne, 1983), and can be released *in vivo* from the adrenal medulla in response to stress (Vaupel *et al.*, 1988). The function of SP and sensory innervation in the adrenal medulla, however, is not known. Studies *in vitro* in bovine chromaffin cells in culture and rat isolated perfused adrenal glands indicate that SP modulates catecholamine (CA) secretion (Boksa & Livett, 1984b; Zhou & Livett, 1987; 1988) so as to maintain catecholamine (CA) output during stress.

Capsaicin is a selective neurotoxin for sensory nerves. When injected into rats as neonates, it causes sensory C-fibres to degenerate, and therefore causes a depletion of SP and other mammalian tachykinins in the sensory nervous system (for review, see Buck & Burks, 1986). Therefore, capsaicin has been widely used as a pharmacological tool to investigate SP function in sensory neurones (for review, see Holzer, 1988). We showed previously that neonatal pretreatment with capsaicin abolished the neuronal component of adrenal CA secretion in response to insulin-induced hypoglycaemia or histamine stress in the anaesthetized rat (Khalil *et al.*, 1986; 1987). These results on anaesthetized rats support our hypothesis that SP-containing sensory fibres innervating the adrenal medulla are required to maintain CA secretion during stress (Khalil *et al.*, 1986; 1987). However, this hypothesis has been challenged by two recent studies.

Firstly, Amann & Lembeck (1986) and Donnerer (1988) proposed that the attenuation of CA secretion in capsaicin-pretreated rats was caused by a failure of peripheral communication to the central nervous system due to the damage of sensory afferent C-fibres. This conflict may be resolved by use of 2-deoxyglucose (2-DG) to produce a state of neuroglucopenia with subsequent adrenal CA secretion (Nijima, 1982; Storlien 1985). 2-DG stimulates glucose-sensitive neurones in the hypothalamus directly, so bypassing the peripheral sensory afferents. However, it still shares the same efferent pathway as insulin-induced hypoglycaemia (Hokfelt & Bydeman, 1961; Brown, 1962; Nijima, 1982) for the release of adrenal CAs.

Secondly, anaesthetics such as pentobarbitone inhibit CA outflow during 2-DG-induced stress (Taborsky *et al.*, 1984a,b; Havel *et al.*, 1987) or hypovolemic stress (Howe *et al.*, 1986). It is possible that the failure of capsaicin-pretreated animals to secrete CAs in response to stress (Khalil *et al.*, 1986; 1987) was a function of the pentobarbitone anaesthesia. In the present study, therefore, we have studied the role of capsaicin-sensitive sensory nerves in the regulation of adrenal CA secretion induced by 2-DG in conscious freely-moving rats fitted with indwelling venous cannulae.

Methods

Animals and surgical preparation

Buffalo rats, male, 10 weeks old (290–380 g), were raised under conditions of constant temperature (21°C) and had free access to standard rat pellets and water. For placement of indwelling cannulae the rats were anaesthetized i.p. with a mixture (0.2 ml

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per 100 g) containing 1% of sodium brietal and 1.5% of sodium amytal (2 ml kg^{-1}). The operation area on the neck and catheter outlet area on the back were shaved and sterilized with 70% ethanol. A longitudinal incision 1.5 cm long was made in the skin on the ventral surface of the neck on the right side. The right jugular vein was exposed by blunt dissection and cannulated with a piece of silastic tubing (i.d. $0.025 \times \text{o.d. } 0.045 \text{ in.}$). A 4 cm length of cannula was inserted into the vein and extended so that the tip of the cannula rested in the inferior vena cava, approximately 1 cm below the right atrium. The cannula was secured into the jugular vein with a cotton tie. The patency of the cannula was tested by withdrawing blood. The free end of the cannula was led subcutaneously to the dorsal surface of the head and externalized through a small incision. The catheter was filled with heparin saline (200 iu ml^{-1}) and plugged with a short stainless steel pin. Finally, the neck incision was sutured and painted with 70% ethanol or Hexifoam. The total operation time was 15–20 min and the animals regained consciousness by 45 min. After operation the animals were housed individually. Thereafter, on a daily basis, the catheters were flushed with heparin saline to maintain catheter patency. The experiments were performed in a quiet laboratory on the 4th day after operation on rats fasted overnight. Before sampling, the rats were heparinized by flushing the catheter with 0.5 ml of heparin saline (200 iu ml^{-1}). A polythene tube (PE-28, i.d. $0.40 \times \text{o.d. } 0.80 \text{ mm}$), which had been connected to a reversible peristaltic pump (Pharmacia P-1) and filled with saline, was connected to the indwelling cannulae. After 10 min (the rat having become quiet and freely moving) sampling was begun by turning on the pump at a rate of 0.5 ml min^{-1} and blood was collected into an ice-cold Eppendorf tube. After each sampling, an equivalent volume of saline was reinfused.

Capsaicin pretreatment Buffalo rat pups of either sex were injected s.c. with either capsaicin (50 mg kg^{-1}) or vehicle under analgesia (Innovar. Vet, Smith Kline and French Laboratories, N.S.W., Australia; dilution 1:100, 10 ml kg^{-1} , s.c.) on the 3rd day after birth as previously described in detail (Khalil *et al.*, 1986). The analgesia was assessed effective as the rat pups remained calm and by the absence of vocalization upon the injection of capsaicin. The solution was prepared as follows; capsaicin 10 mg was dissolved in 0.2 ml ethanol and 0.2 ml Tween-80 and then mixed with 1.6 ml 0.9% NaCl. The survival rate of capsaicin-injected pups was the same as for vehicle-injected pups and non-injected pups. When the rats were 10 weeks old the males were selected for the studies described below.

Left splanchnicotomy and right adrenalectomy Complete denervation of the splanchnic nerve was performed in adult male Buffalo rats (290–380 g) as described previously in detail (Khalil *et al.*, 1987). Briefly, under anaesthesia with Equithesin ($0.2 \text{ ml } 100 \text{ g}^{-1}$ body weight; Equithesin is a pentobarbitone sodium/chloral hydrate mixture in MgSO_4 , propylene glycol and ethanol, see Tagerud & Cuello, 1979), an L-shaped incision was made on the left side of the abdomen. The left splanchnic nerve trunk under the diaphragm was cut and adipose tissue surrounding the left adrenal gland was dissected from the gland. The abdomen was closed and the rats allowed to recover in a warm environment and monitored for 4 h after the operation by which time they had fully recovered from anaesthesia. They were then transferred to separate cages in an air-conditioned room with free access to food and water. The site of incision healed without infection in all cases. These operated rats were used for experiments after two weeks. Right adrenalectomy was carried out on these rats with left splanchnicotomy at the same time as the preparation of the indwelling catheter (see above). A 2-cm-long incision was made on the right abdomen and the right adrenal gland removed. The operation for removal of the right adrenal gland was completed in 5 min.

Assay of catecholamines and plasma glucose

Blood samples were centrifuged at $4000 g$ for 15 min, 2°C . The plasma was separated and transferred into Eppendorf tubes containing $30 \mu\text{l}$ sodium metabisulphate (10%) and stored at -70°C until assay. For assay of adrenaline (Ad) and nor-adrenaline (NA), CAs were extracted from the plasma on acid-washed alumina and eluted with 0.2 M perchloric acid (PCA). The extracted samples were injected onto a high performance liquid chromatography (h.p.l.c.) system with electrochemical detection as described by Livett *et al.* (1987). The recovery was corrected by adding an internal standard dihydroxybenzylamine (DHBA) to the extraction buffer. The content of CA in plasma was calculated as pmol ml^{-1} .

Plasma glucose was estimated by the hexokinase method (Glucocuant, Boehringer, Mannheim, FRG). 2-DG could not be detected in concentrations lower than 27 mmol l^{-1} with this method.

Adrenal medullary catecholamine determination

Adult male Buffalo rats (10 weeks old, 290–380 g) from the vehicle- and capsaicin-pretreatment groups were killed by stunning and decapitation and the adrenal glands were removed and homogenized in 2 ml 0.4 M PCA. The supernatant after centrifugation was injected into the h.p.l.c. system directly.

Experimental procedures

Experiments in vivo on conscious freely-moving rats Rats were fasted overnight before the experiments. After the experiments, the rats were given free access to food and water, until killed. Each animal was used only for a single blood sampling experiment.

Effects of 2-DG on CA secretion Four groups of rats ($n = 5$ in each group) were used. *Group 1:* After withdrawal of a resting blood sample, 2-DG (500 mg kg^{-1}) was infused i.v. over a 5 s period. Blood samples were withdrawn 4 times thereafter, at 15 min intervals. *Group 2:* After withdrawal of a basal sample, hexamethonium (2 mg kg^{-1}) and atropine (1 mg kg^{-1}) in normal saline were infused i.v. before and 30 min after 2-DG. Blood sampling was as in group 1. *Group 3:* After withdrawal of a basal sample, pentolinium (2 mg kg^{-1}) was infused i.v. before and 30 min after 2-DG. Blood sampling was as in Group 1. *Group 4:* Rats with right adrenal glands removed and left splanchnic nerve cut were given 2-DG and samples taken for plasma glucose and plasma CAs as described for Group 1. Animals in Group 4 were subjected to a separate series of experiments on the next day in which the left denervated adrenal glands were isolated and stimulated *in vitro* to confirm that the denervation was successful (see below).

Experiments in vitro on isolated perfused denervated adrenal glands On the day following blood sampling *in vivo*, rats from group 4 above were anaesthetized with Equithesin ($0.2 \text{ ml } 100 \text{ g}^{-1}$, i.p.), the left adrenal vein cannulated with PE-10 tubing and the adrenal gland was isolated and perfused with Krebs bicarbonate solution. After 20 min equilibration the adrenal gland was subjected to electrical stimulation with a Grass S48 stimulator (60 V, 10 Hz and 3 ms) for 2 min and the perfusate collected for CA determination. After a 10 min interval, the gland was perfused with 0.1 mM dimethylphenylpiperazinium iodide (DMPP) for 2 min and the perfusate collected for CA determination. As controls, adrenal glands from 5 non-denervated rats were prepared for *in vitro* perfusion.

Experiments on capsaicin pretreated rats Two sampling procedures were used *in vivo* on capsaicin-pretreated rats and

vehicle-pretreated rats. In one protocol blood samples were withdrawn at 0, 15, 30, 45, and 60 min after 2-DG and adrenal glands were removed for CA assay after 24 h. With the other protocol, blood samples were withdrawn at 0, 5, 60, 90, and 120 min after 2-DG and the adrenal glands were removed for CA assay after 8 h. Data from two protocols were plotted together. To study the effect of 2-DG on tissue levels of adrenal CA, five capsaicin- and five vehicle-pretreated rats without blood sampling and without using 2-DG treatment were operated on as for insertion of the venous cannula and served as sham-operated controls.

Materials

Hexamethonium, atropine sulphate, capsaicin and pentolinium were obtained from Sigma Chemical Co, U.S.A.; 2-deoxyglucose was obtained from Aldrich Chemical Co, U.S.A.; Silastic tubing was from Dow Corning Corporation, U.S.A.

Statistical analysis

Data are expressed by means $\pm$ s.e.mean. Significant differences were assessed by analysis of variance and Student's *t* test.

Results

Plasma glucose levels during 2-deoxyglucose-induced stress

The resting plasma glucose level in rats after an overnight fast was 102 ± 5 mg%. After 2-DG (500 mg kg^{-1} , i.v.), the plasma glucose level increased to 189 ± 13 mg% at 15 min and to 250 ± 37 mg% at 30 min and then levelled off. Ganglionic blockade with hexamethonium and atropine had no significant effects on plasma glucose levels after 2-DG (Figure 1a), whereas pentolinium significantly attenuated the increase in plasma glucose by 50% ($P < 0.01$) at 30, 45, and 60 min after 2-DG. Plasma glucose levels following 2-DG in rats with their right adrenal glands removed and left splanchnic nerves cut were significantly lower than in the control animals at 30 ($P < 0.05$), 45 ($P < 0.01$), and 60 ($P < 0.05$) min.

Plasma catecholamine levels in 2-deoxyglucose-induced stress

The resting i.v.c. plasma levels of NA and Ad before 2-DG were 6.8 ± 1.1 and $5.7 \pm 0.8 \text{ pmol ml}^{-1}$. Plasma NA levels after 2-DG increased gradually up to $24.1 \pm 2.8 \text{ pmol ml}^{-1}$ at 60 min ($P < 0.01$ at all the points compared to resting level). Ad levels increased to $39.7 \pm 8.1 \text{ pmol ml}^{-1}$ at 30 min and then levelled off at 45 and 60 min after 2-DG ($P < 0.01$ at all time points compared to the resting level). As Figure 1 shows, the CA response to 2-DG was significantly suppressed but not blocked by the treatment with hexamethonium and atropine ($P < 0.05$ at 45 and 60 min for NA and $P < 0.01$ at 45 and 60 min for Ad, compared with 2-DG alone). However, pentolinium at 2 mg kg^{-1} completely blocked the increase in plasma CA in response to 2-DG (Figure 1). The Ad response to 2-DG was completely abolished and NA response to 2-DG was substantially inhibited in the rats with their right adrenal gland removed and left splanchnic nerve denervated (Figure 1).

Catecholamine secretion from denervated glands in vitro

CA secretion evoked by electrical field stimulation of isolated perfused glands taken from rats which had undergone surgical adrenal denervation was almost completely abolished (Figure

Figure 1 Effect of 2-deoxyglucose (2-DG) on plasma glucose and catecholamine (CA) levels in conscious rats. After withdrawal of basal blood samples, 2-DG (500 mg kg^{-1}) was infused i.v. and blood was sampled every 15 min from the inferior vena cava for CA and plasma glucose assay. (a) Plasma glucose; (b) noradrenaline; (c) adrenaline. (○) 2-DG only; (●) 2-DG + hexamethonium (2 mg kg^{-1}) + atropine (1 mg kg^{-1}); (△) 2-DG experiments in the animals with right adrenal glands removed and left splanchnic nerves denervated; (▲) 2-DG + pentolinium (2 mg kg^{-1}). * $P < 0.05$; ** $P < 0.01$ compared with 2-DG only (○). Data are plotted as means with s.e.mean shown by vertical bars ($n = 5$). Where error bars are not visible they lay within the dimensions of the symbol.

2). However, CA secretion from these glands in response to the nicotinic agonist DMPP was unaffected (Figure 2). Glands from control animals responded well to both nerve and drug stimulation.

Figure 2 Effect of surgical adrenal denervation on adrenaline secretion evoked by field stimulation of isolated perfused adrenal gland. Two weeks after left splanchnicotomies and one day after the 2-deoxyglucose experiment, the left adrenal glands were isolated, perfused and stimulated at 10 Hz with 60 V and 3 ms pulse as described in the text. Open columns: control glands from non-denervated rats; hatched columns: denervated glands. DMPP: dimethylphenylpiperazinium iodide. Data are plotted as means with s.e.mean shown by vertical bars ($n = 4$).

Figure 3 Effect of capsaicin pretreatment on plasma glucose and catecholamine levels in response to 2-deoxyglucose (2-DG) in conscious rats. The rats were treated with either 50 mg kg^{-1} capsaicin (s.c.) or with vehicle on the third day after birth. Stress experiments were performed when rats were 8–12 weeks old. After withdrawal of the basal blood samples, 2-DG 500 mg kg^{-1} was infused i.v. and blood samples were withdrawn at the times shown in the figure. (a) Plasma glucose; (b) noradrenaline; (c) adrenaline. (○) Vehicle control rats; (●) capsaicin-pretreated rats. * $P < 0.05$ compared with vehicle-pretreated rats. Data are plotted as means with s.e.mean shown by vertical bars ($n = 10$ at 0 and 60 min and $n = 5$ at 5, 15, 45, 90 and 120 min).

Effect of capsaicin pretreatment on plasma glucose levels after 2-deoxyglucose

There was no significant difference in the resting plasma glucose levels between capsaicin- and vehicle-pretreated rats (Figure 3a, $t = 0$). In the first 30 min after 2-DG, plasma glucose levels in capsaicin-pretreated rats increased to the same levels as in vehicle-pretreated rats, whereas at 45, 60, and 90 min, the plasma glucose levels in capsaicin-pretreated rats were significantly attenuated ($P < 0.05$ compared with the levels in vehicle-pretreated rats). By 120 min, there was again no significant difference in the plasma glucose levels between the two groups (Figure 3a).

Effect of capsaicin pretreatment on plasma catecholamine levels after 2-deoxyglucose

Plasma CA levels in response to 2-DG in both capsaicin- and vehicle-pretreated rats exhibited substantial individual variation. Plasma Ad levels in some rats increased up to 150 pmol ml^{-1} , whereas the increase was much less in other rats. The time course of plasma CA changes after 2-DG also exhibited large variation between individuals of same treatment group. The increment in plasma CA levels in some rats peaked earlier, some later. On average, plasma NA and Ad

Figure 4 Effect of 2-deoxyglucose (2-DG) on tissue catecholamine (CA) levels in the adrenal gland. 2-DG (500 mg kg^{-1}) was infused i.v. to conscious rats pretreated with capsaicin or vehicle as neonates. The rats were killed at 0 (sham-operated group, without 2-DG), 8 and 24 h after 2-DG and CA levels in the adrenal medulla were measured. (a) Left adrenal glands; (b) right adrenal glands. (○) Adrenaline (Ad) vehicle; (●) Ad capsaicin; (△) noradrenaline (NA) vehicle; (▲) NA capsaicin. ** $P < 0.01$ compared with vehicle-pretreated rats. Data are plotted as means with s.e.mean shown by vertical bars ($n = 5$ at each point).

levels started to increase at 5 min in both groups and peaked at 30–45 min, and declined thereafter. After 60 min, plasma Ad levels in the capsaicin-pretreated rats were still high and at 90 and 120 min approximately twice that in the vehicle pretreated rats ($P < 0.05$ at 90 and 120 min) (Figure 3c). There was no significant effect of capsaicin pretreatment on plasma NA levels (Figure 3b).

Effect of capsaicin pretreatment on adrenal catecholamine concentrations

Eight hours after treatment with 2-DG, Ad concentrations in the left adrenal glands of vehicle-pretreated rats were depleted by 88.4% and in the capsaicin-pretreated rats by 84.6% (Figure 4). NA concentrations in the left adrenal glands of capsaicin- and vehicle-pretreated rats were depleted by only 21.3% and 33.4% respectively. There were no significant differences between capsaicin and vehicle pretreated groups 8 h after 2-DG.

The tissue CA levels recovered faster over 24 h in the capsaicin group (from 10.8 to $26.5 \text{ nmol per gland}$) than in the vehicle group (from 6.2 to $10.0 \text{ nmol per gland}$) (Figure 4). The tissue levels of Ad in the adrenals of the capsaicin group after 24 h were significantly higher than those in the vehicle group ($P < 0.01$). The adrenal NA levels in both groups after 24 h not only recovered but also increased above the fasting control levels. The changes in CA levels in the right adrenal gland after 2-DG were similar to those in the left adrenal gland.

Discussion

Effect of 2-deoxyglucose on plasma glucose levels

The results of the present study confirmed that 2-DG administration induces hyperglycaemia and secretion of Ad from the

adrenal medulla. We found that the ganglion blocker pentolinium partially blocked the hyperglycaemia but completely blocked the increase in adrenal CA secretion. Similarly, right adrenalectomy plus left splanchnicotomy partially blocked the hyperglycaemia but completely blocked the increase in adrenal CA secretion (Figure 1).

The mechanism of the hyperglycaemic response to 2-DG is not fully understood. The compound 2-deoxyglucose (2-DG), is a non-metabolizable D-glucose analogue. When administered systemically or intraventricularly, it causes depletion of glucose within cells (glucopenia) (Wick *et al.*, 1955) and brings about a neurogenic activation of glucose-sensitive neurones in the hypothalamus with increased sympathetic outflow and consequent hyperglycaemia (Muller *et al.*, 1971), and CA secretion (Hokfelt & Bydeman, 1961; Himsworth, 1979; Taborsky *et al.*, 1984b; Donnerer, 1988) from the adrenal medulla. It is believed that the hypothalamus (Muller *et al.*, 1971; Storlien, 1985), and central (Nijima, 1982) and peripheral sympathetic pathways (Nijima, 1975) and the adrenal medulla but not the sensory afferents (Storlien, 1985; Amann & Lembeck, 1986) are involved in this pathway. Earlier studies demonstrate that adrenalectomy and adrenal denervation inhibit the hyperglycaemic response to 2-DG (Brown, 1962; Frohman *et al.*, 1983), indicating that it is mediated by the adrenal medulla. Studies by Storlien *et al.* (1985) show that the 2-DG-induced hyperglycaemia is blocked by pretreatment with guanethidine, an adrenergic nerve blocker, suggesting that the hyperglycaemic response to 2-DG is mediated by sympathetic nerves innervating the liver rather than by adrenaline released from the adrenal gland. Our results in the present study indicate that cholinergic secretion of Ad from the adrenal medulla is not solely responsible for the 2-DG-induced hyperglycaemia. In this regard, it is known that 2-DG increases glucagon (Frohman & Bernadis, 1971) and pancreatic polypeptide (PP) (Taborsky *et al.*, 1984a) secretion and inhibits insulin secretion. These pancreatic endocrine hormones may therefore contribute to the non-cholinergic component of 2-DG-induced hyperglycaemia.

Effect of capsaicin pretreatment on plasma glucose levels

In the present study, we found that the hyperglycaemia in response to 2-DG was attenuated by capsaicin pretreatment. This result is in accordance with results obtained with insulin-induced hypoglycaemia in which the recovery of blood glucose levels after insulin was impaired in capsaicin pretreated rats (Zhou *et al.*, 1990). The reason for this dysfunction in glucose homeostasis is not clear. The secretion of the counter-regulatory hormones, adrenaline and glucagon in response to 2-DG, might be impaired in the absence of sensory fibres. However, Ad secretion in response to 2-DG in capsaicin-pretreated rats was not impaired (Figure 3c), being even higher than in vehicle-pretreated rats. This indicates that the attenuation in hyperglycaemia in capsaicin-pretreated rats is not caused by impairment of CA secretion. Thus the attenuation of hyperglycaemia may have been caused by dysfunction of glucagon secretion. Glucagon is the primary counter-regulatory hormone (Gerich *et al.*, 1979) and its secretion is increased in response to 2-DG administration (Hedo *et al.*, 1978; Frohman *et al.*, 1983). SP infusion *in vivo* induces hyperglucagonaemia (Brown & Vale, 1976; Lundquist *et al.*, 1979), suggesting depletion of SP by capsaicin pretreatment may cause dysfunction of glucagon secretion. In addition, the impairment of adrenal cortical hormone secretion in capsaicin-pretreated animals (Lembeck & Amann, 1986; Donnerer & Lembeck, 1988) may also contribute to the attenuation of the blood glucose response to 2-DG.

Effect of 2-deoxyglucose on plasma catecholamine levels

The basal levels of plasma CAs were higher in the present study than those reported by others (Taborsky *et al.*, 1984a; Howe *et al.*, 1986). The difference may be explained by the fact

that we sampled blood from the inferior vena cava close to the point of venous output from the adrenal gland. In fact one of the advantages of our sampling technique is that it overcomes the problem of high clearance of blood-borne CAs that occurs on passing through tissue vascular beds. Despite widespread use of plasma CAs in the clinical literature, peripheral venous plasma Ad concentrations are a very poor substitute for central venous or arterial concentrations.

2-DG induced a marked increase in plasma CA levels (Figures 1 and 3) with an approximately 90% depletion in tissue Ad levels in the adrenal glands (Figure 4). To investigate the mechanism involved in the initial CA secretion induced by 2-DG, we used hexamethonium (C_6) and atropine to see whether the CA response to 2-DG was under cholinergic control. The results showed that C_6 and atropine did not completely block the CA response. Two possible explanations for these results were: (1) that there were non-cholinergic splanchnic nerve components contributing to the control of adrenal CA secretion after 2-DG; and that (2) C_6 and atropine at all concentrations used were not effective in blocking ganglionic transmission in the conscious rat. Consequently, pentolinium was used to produce a potent ganglion blockade in the conscious rat (Howe *et al.*, 1986). Pentolinium completely blocked CA secretion after 2-DG, indicating that the CA response to 2-DG was controlled by the cholinergic nervous system and that C_6 at 2 mg kg^{-1} and atropine at 1 mg kg^{-1} were not effective blockers of the cholinergic response in conscious rats.

To study the source of CA secretion in response to 2-DG, we cut the left splanchnic nerve and removed the right adrenal gland before the release experiment. The results showed that CA secretion was completely blocked in the rats with the left splanchnic nerves denervated and the right adrenal glands removed (Figure 1), indicating that the increase in plasma CA in response to 2-DG came almost entirely from the adrenal medulla rather than from sympathetic nerve terminals.

The results in Figure 2 indicate that the procedure used to denervate the adrenal medulla by cutting the splanchnic nerves was successful as shown by an almost complete lack of CA secretion in response to electrical field stimulation of such glands *in vitro*. The secretory function of the adrenal chromaffin cells remained intact, however, because a full CA secretory response was elicited by the nicotinic agonist DMPP.

Effect of capsaicin pretreatment on catecholamine secretion

Numerous studies on a variety of systems have shown that CA secretion in response to nicotinic stimulation is very quickly desensitized *in vitro* (Boksa & Livett, 1984a). That desensitization does not occur *in vivo* even during severe stress suggests that there are physiological mechanisms preventing desensitization of the nicotinic response. Our previous work with bovine adrenal chromaffin cells (Boksa & Livett, 1984b) has shown that SP functions to protect against desensitization of the nicotinic response induced by either acetylcholine or nicotine. More recently, we have shown that SP at low concentrations ($< 10^{-6} \text{ M}$) facilitates CA secretion evoked by short term (6 min) or long term (2 h) electrical field stimulation of the isolated perfused adrenal glands of the rat (Zhou & Livett, 1987; 1988). These *in vitro* studies suggest that SP may act to prevent desensitization of the nicotinic response during stress *in vivo*. In addition, capsaicin-pretreatment, which depletes SP from the splanchnic nerve innervating the adrenal medulla, abolished CA secretion in response to insulin-induced hypoglycaemic stress, cold stress (Khalil *et al.*, 1986) and the neuronal component of histamine-induced stress (Khalil *et al.*, 1987) in anaesthetized rats. However, in the present study, CA secretion in response to 2-DG-induced glucopenia occurred at the same initial rate in capsaicin-pretreated rats as in the vehicle-pretreated rats. At later times, this response in the capsaicin-pretreated rats was even higher than in the vehicle-pretreated rats (Figure 3b,c). CA depletion

in the adrenal medulla 8 h after 2-DG in capsaicin pretreated rats was not significantly different from that in vehicle-pretreated rats. There are three possible explanations for the difference in results between the present study and our previous studies: (1) Sensory afferents of reflex arcs which can be damaged by capsaicin pretreatment are involved in the stimuli used in the previous studies; (2) Pentobarbitone used in the previous studies may suppress CA output during stress (Taborsky *et al.*, 1984a,b; Howe *et al.*, 1986; Havel *et al.*, 1987); (3) Greater blood sample withdrawal in the previous studies (7.5 ml vs 5 ml in the present study) may have resulted in hypovolemia which added to the three stimuli employed (insulin stress, cold stress and histamine stress).

By using 2-DG directly to stimulate central sympathetic outflow in the present study in freely moving conscious rats, we excluded any possible involvement of sensory afferents in the activation of CA secretion from the adrenal medulla by a reflex pathway and effects of anaesthetics on CA secretion. We also reduced the blood sample volume to avoid hypovolemic stress. The results of the present study therefore, demonstrate that in the freely-moving conscious rats, SP-containing capsaicin-sensitive sensory fibres innervating the adrenal medulla are not required to maintain CA secretion during prolonged glucopenic stress. They are, however, involved in the glucose homeostasis in conscious animals. A similar conclusion was reached from studies on the effects of insulin/somatostatin-induced hypoglycaemia on CA release from

capsaicin-pretreated and vehicle-pretreated conscious rats sampled *in vivo* via indwelling cannulae (Zhou *et al.*, 1990).

In conclusion, capsaicin-sensitive sensory fibres innervating the adrenal gland appear not to be required for activation and maintenance of CA secretion during prolonged glucopenic stress in the conscious freely-moving rat. The mechanism demonstrated in isolated chromaffin cells (Boksa & Livett, 1984b), in the rat perfused adrenals *in vitro* (Zhou & Livett, 1987; 1988) and in the anaesthetized rat (Khalil *et al.*, 1986; 1987) in which endogenous substance P serves to modulate the nicotinic secretion of catecholamines in response to acetylcholine, nicotinic agonists, electrical stimulation and a number of metabolic and physical stimuli appears to be subservient to control mechanisms operating at higher levels in the conscious animal. Nevertheless, CA secretion in the conscious animal does not exhibit desensitization in response to physiological stress. The mechanisms responsible for preventing desensitization of the CA secretory response during stress *in vivo* remain to be determined.

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