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RENIN-ANGIOTENSIN MEDIATION OF ADRENAL CATECHOLAMINE SECRETION INDUCED BY HYPOGLYCAEMIA IN THE CAT

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1 The mechanism involved in catecholamine (CA) release from the cat adrenal gland in response to insulin hypoglycaemia was studied. In intact cats, hypoglycaemia induced an 11 fold increase in adrenomedullary CA secretion.

2 Acute bilateral nephrectomy nearly abolished the increased CA release from the adrenal gland during hypoglycaemia.

3 Infusion of Sar^1-Ileu^8-Angiotensin II (AII), a competitive AII antagonist, suppressed the adrenomedullary response to the insulin-induced hypoglycaemia. After termination of the antagonist infusion, CA secretion from the adrenal medulla increased rapidly, reaching the same level as in insulin-treated cats.

4 Infusion of rabbit anti-angiotensin I antibodies suppressed CA release from the adrenal gland of hypoglycaemic cats. This effect was more prolonged than that of Sar^1-Ileu^8-AII.

5 These results indicate that CA release from the adrenal medulla of the cat in response to insulin-induced hypoglycaemia, is mediated through the renal reninangiotensin system. Since hypoglycaemia causes sympathetic stimulation through a central mechanism, angiotensin may act through the central nervous system.

Introduction

Increased sympathetic activity caused by hypoglycaemia was first described by Cannon, McIver & Bliss (1924) in cats and later confirmed in man and other species by several authors (Goldfien, Moore, Zileli, Hevens, Boling & Thorn, 1961; Himms-Hagen, 1967; Garber, Cryer, Santiago, Hammond, Pagliara & Kipnis, 1976). The sympathetic activation by hypoglycaemia was shown to occur through the central nervous system (Crone, 1965; Otsuka, Assaykeen, Goldfien & Ganong, 1970), but peripheral humoral factors have also been implicated in the adrenergic response to insulin hypoglycaemia (Scian, Westerman, Verdesca & Hilton, 1960). Since angiotensin causes release of catecholamines (CA) both from adrenergic nerve terminals (Reit, 1972) and from the adrenal medulla (Ackerly, Sarstedt & Peach, 1977), it is reasonable to assume that this hormone is also involved in hypoglycaemia-induced CA release. Recently, we have reported that, in the cat, adrenomedullary response to acute haemorrhage is mediated by the renal renin-angiotensin system (Feuerstein, Boonyaviroj, Gutman, Khosla & Bumpus, 1977a; Feuerstein, Boonyaviroj & Gutman, 1977b). Insulin hypoglycaemia causes increased release of CA (Feuerstein & Gutman, 1971), activation of the renin-angiotensin system (Otsuka *et al.*, 1970; Lowder, Frazer & Liddle, 1975) and adrenergic stimulation (Hedeland, Dymling & Hökfelt, 1972; Hökfelt, Hansson, Heding & Nilsson, 1978). CA also stimulate release of renin (Zanchetti & Stella, 1975; Ganong & Reid, 1976), and it seemed, therefore, of interest to determine whether the primary event during hypoglycaemia is CA secretion or release of renin.

Methods

Male cats, weighing 2.5 to 4.0 kg, were anaesthetized with pentobarbitone sodium (50 mg/kg, i.m.), and the femoral artery and vein were cannulated. The left adrenolumbar vein was cannulated for adrenal venous blood sampling. Blood was collected at 10 min intervals over a period of 120 min. In all experiments, three consecutive samples of adrenal venous blood were taken before induction of hypoglycaemia. The mean CA secretion rate of the second and third of

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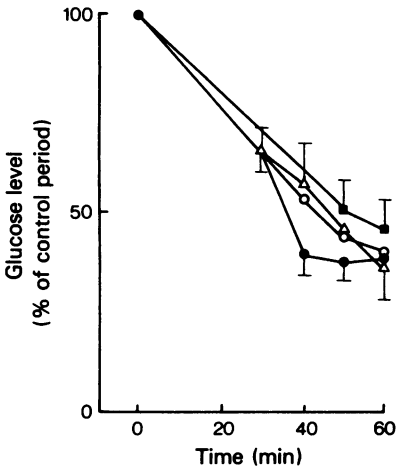

Figure 1 Effect of insulin injection (i.v.) on blood glucose levels in anaesthetized cats: (●) control cats, injected with insulin (10 u/kg; $n = 9$); (○) Sar^1-Ileu^8-angiotensin II-treated cats with insulin hypoglycaemia ($n = 6$); (△) nephrectomized cats, exposed to insulin hypoglycaemia ($n = 6$); (■) anti-angiotensin I antibodies injected into cats with insulin hypoglycaemia ($n = 4$). Vertical bars show s.e. in all figures. Insulin was injected at time zero.

these samples served as control. Subsequent samples were compared to the control samples of the same animal, thus enabling evaluation of the responses in animals with widely varying basal CA secretion rates.

Hypoglycaemia was induced by intravenous injection of 10 u/kg insulin (Insulin Leo, Nordisk, Copenhagen, Denmark). Arterial blood samples (1.0 ml) were collected throughout the experiment for blood glucose determination by the glucose oxidase test (Hjelm & de Verdier, 1963).

Bilateral nephrectomy was performed by double ligation of the renal blood vessels and the ureter at the hilus of the kidneys without removal of the kidneys.

Infusion of Sar^1-Ileu^8-AII

Sar^1-Ileu^8-AII (synthesized by M. C. Khosla and M. F. Burnbur, Research Division, Cleveland Clinic, Cleveland, Ohio, U.S.A.) was dissolved in 0.9% w/v NaCl solution (50 μg in 10 ml) and infused intravenously (0.4 μg kg^{-1} min^{-1}) for 40 min, starting at the point of insulin injection.

Infusion of rabbit anti-angiotensin I antibodies

Anti-antiotensin I antibodies were produced according to the method described by Goodfriend, Levine & Fasman, (1964). Rabbit plasma (titre > 1:5,000) was infused at a rate of 0.1 ml kg^{-1} min^{-1}, starting 5 min after the insulin injection and continued for 30 min. Normal rabbit plasma, infused following the insulin injection, had no effect on CA response to hypoglycaemia.

Isolation and assay of Catecholamines

Adrenal venous blood samples were collected into ice-cold test tubes containing 1.0% disodium edetate plus 1% ascorbic acid. The plasma was separated by centrifugation (5 min at 4,000 *g* at 4°C). Protein was precipitated by acidification with 4 M perchloric acid (1/10: v/v), followed by centrifugation at 7,000 *g* for 10 min at 4°C.

Adrenaline and noradrenaline were assayed by the trihydroxyindole method, after isolation on aluminium oxide columns, followed by passage through Bio-Rex 70 columns, as described previously (Feuerstein *et al.*, 1977b). From the values of adrenaline and noradrenaline the total amount of catecholamines (CA) was calculated. The results are expressed as amount of CA (in ng) released into the adrenolumbar vein per kg body wt during each period of collection, of 10 min (ng kg^{-1} 10 min^{-1}).

Results

Effect of insulin injection on plasma glucose levels

Insulin induced marked hypoglycaemia, with blood glucose levels falling to less than 50% of control values in all cats (Figure 1). The hypoglycaemia was not statistically different in the various experimental groups (intact cats, nephrectomized cats, and cats treated with the AII antagonist or with antibodies to angiotensin I). Blood glucose of cats cannulated as the experimental cats but not injected with insulin and anaesthetized for the same period as the experimental cats, was 107 ± 4 mg% ($n = 8$) at the beginning of the experiments, and no significant deviation occurred throughout the entire experiment.

Effect of nephrectomy on adrenal catecholamine response to hypoglycaemia

In intact cats, the CA secretion rate was 50 ± 7 ng kg^{-1} 10 min^{-1} during the control period. Ten minutes after insulin administration, CA secretion increased to 161 ± 44 ng kg^{-1} 10 min^{-1} ($P < 0.05$), and 70 min following insulin it reached 465 ± 109 ng kg^{-1} 10 min^{-1} ($P < 0.001$). A predominance of adrenaline was observed in the CA secreted in response to hypoglycaemia, as previously reported (Feuerstein & Gutman, 1971). In bilaterally nephrectomized cats,

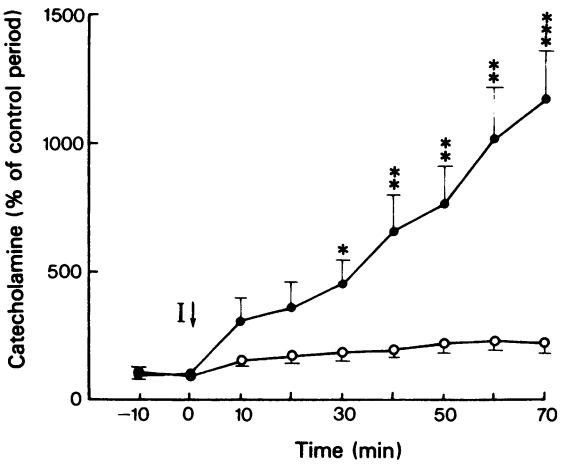

Figure 2 Catecholamine release from the adrenal medulla of the cat in response to insulin hypoglycaemia. Ordinate scale: catecholamine secretion as % of secretion rate during control period: (●) control cats injected with insulin (10 u/kg; $n = 9$); (○) bilaterally nephrectomized cats injected with insulin (10 u/kg; $n = 6$); I ↓ indicates time of insulin injection. $*P < 0.02$; $**P < 0.01$; $***P < 0.001$ for differences between control and experimental groups (in all figures).

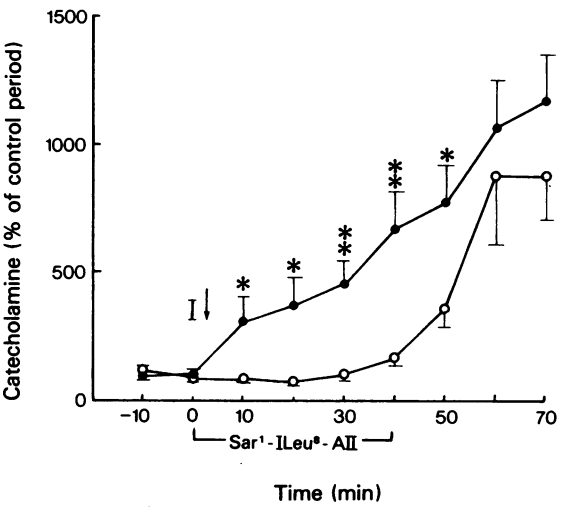

Figure 3 Effect of angiotensin antagonist on catecholamine release from the adrenal gland of the cat in response to hypoglycaemia: (●) control cats injected with insulin (10 u/kg; $n = 9$); (○) experimental group, cats administered insulin (10 u/kg and Sar¹-Ileu⁸-angiotensin II ($n = 6$)); the period of Sar¹-Ileu⁸-angiotensin II infusion (0.4 $\mu g\,kg^{-1}\,min^{-1}$) is indicated. I ↓ indicates time of insulin injection. $*P < 0.05$; $**P < 0.01$.

basal CA output was $52 \pm 4\,ng\,kg^{-1}\,10\,min^{-1}$, but the response to insulin was considerably attenuated (Figure 2); a significant increase in CA secretion was observed only after 40 min following the insulin injection, $103 \pm 20\,ng\,kg^{-1}\,10\,min^{-1}$ ($P < 0.05$), while no further elevation in the CA secretion rate was detected throughout the remainder of the experiment.

Effect of angiotensin II antagonist on adrenal catecholamine respone to hypoglycaemia

During continuous infusion of the A II antagonist, no significant increase in CA secretion was found following insulin. Forty min after insulin administration, when glucose levels had decreased to 50% of the control level, CA secretion was $84 \pm 20\,ng\,kg^{-1}\,10\,min^{-1}$, as compared to $51 \pm 11\,ng\,kg^{-1}\,10\,min^{-1}$ during the control period (difference not statistically significant). In the control group (without AII antagonist), CA secretion 40 min following insulin was significantly higher than in the AII antagonist-treated group (Figure 3). Once the AII antagonist infusion was stopped, a rapid increase in CA secretion occurred from $84 \pm 20\,ng\,kg^{-1}\,10\,min^{-1}$ at the end of the infusion period to $227 \pm 75\,ng\,kg^{-1}\,10\,min^{-1}$, and $457 \pm 140\,ng\,kg^{-1}\,10\,min^{-1}$, 10 and 20 min later, respectively. Thus, 20 min after termination of the AII antagonist infusion, the adrenal CA secretion rate was of the same magnitude in both the control and experimental group. Thus Sar¹-Ileu⁸-AII suppressed CA secretion only for the duration of the infusion of the antagonist.

Effect of antibodies to angiotensin I on adrenal catecholamine response to hypoglycaemia

Infusion of antibodies to angiotensin I suppressed the adreno-medullary response to insulin-induced hypoglycaemia (Figure 4); at the end of the infusion, CA secretion was $73 \pm 24\,ng\,kg^{-1}\,10\,min^{-1}$, as compared to $56 \pm 13\,ng\,kg^{-1}\,10\,min^{-1}$ during the control period (difference not statistically significant). During this period, CA secretion in the control group (insulin alone) was significantly higher. Suppression of CA secretion by anti-angiotensin I antibodies continued beyond the infusion period.

Discussion

Hypoglycaemia increases the circulating levels of several hormones, some of which, CA (Kneer, Bosch, Clark & Lardy, 1974), glucagon (Clark, Kneer, Bosch & Lardy, 1974) and cortisol (Exton, 1972), directly

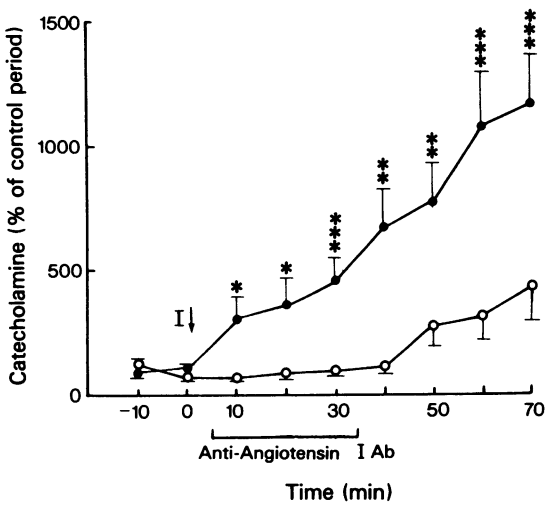

Figure 4 Effect of anti-angiotensin I antibodies on catecholamine release from cat adrenal medulla in response to hypoglycaemia: (●) control cats injected with insulin (10 u/kg; $n = 9$); (○) experimental group, cats administered insulin (10 u/kg; $n = 4$) and angiotensin I antibodies; the period of angiotensin I antibodies (Ab) infusion (0.1 ml kg^{-1} min^{-1}) is indicated; I ↓ Indicates time of insulin injection. $*P < 0.05$; $**P < 0.02$; $***P < 0.001$.

contribute to the production or release of glucose, while others, such as growth hormone, decrease glucose utilization (Fineberg & Merimee, 1974). Although elevated plasma renin activity during insulin hypoglycaemia has been described (Lowder *et al.*, 1975), this finding was not further explored since AII has no significant effect on glucose metabolism.

The experiments described here indicate that the renal renin-angiotensin system does play a major role in mediation of the adrenomedullary response to hypoglycaemia, i.e. activation of the former is obligatory for full adrenomedullary response to profound hypoglycaemia. This phenomenon is similar to the abolition of the adrenal CA response to haemorrhage when renin release was suppressed or completely eliminated (Feuerstein *et al.*, 1977a, b).

Hypoglycaemia-induced adrenal CA secretion has been shown to act via the central nervous system (Crone, 1965; Otsuka *et al.*, 1970). It is, therefore, suggested that AII production, stimulated by hypoglycaemia, causes adrenal CA secretion through a central nervous system effect. AII has been found to act on several nuclei of the brain stem (Keim & Sigg, 1971; Buckley & Jandhyala, 1977). It would seem possible, therefore, that AII also modulates central sympathetic centres that are activated by hypoglycaemia. It is noteworthy that electrical stimulation of specific areas in the brain stem has been shown to induce secretion of renin (Passo, Assaykeen, Otsuka & Wise, 1971; Richardson, Stella, Leonetti, Bartorelli & Zanchetti, 1974).

Species differences for the regulation of blood pressure in anephric rats and cats have been described (Harrison, Seaton & Bartlett, 1966, and unpublished data from our laboratory). It was of interest therefore, to determine whether the effect of AII on adrenal CA secretion in response to hypoglycaemia occurs uniquely in the cat, or whether it is a more general phenomenon. Preliminary results (data to be published) showed a significant depletion of the adrenal CA 4 h after insulin-induced hypoglycaemia in nephrectomized rats. This would indicate that the adrenomedullary response to hypoglycaemia in the rat is not mediated through AII.

Whether an interdependence between the renin-angiotensin and the adrenergic nervous systems during hypoglycaemia also exists in man is still uncertain. Some clinical data suggest that hypoglycaemia may be more dangerous in diabetic and non-diabetic patients suffering from renal insufficiency (Rabau, Dor, Asar, Walden & Moses, 1973; Dua, 1974). This phenomenon has been attributed to delayed endogenous or exogenous insulin degradation (O'Brien & Sharpe, 1965), or to impairment of carbohydrate metabolism (Cohen, 1962). The data presented in the present paper suggest that impairment of adrenal CA secretion due to insufficient renin secretion could be a contributory factor to hypersensitivity to insulin-induced hypoglycaemia in patients suffering from renal insufficiency.

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