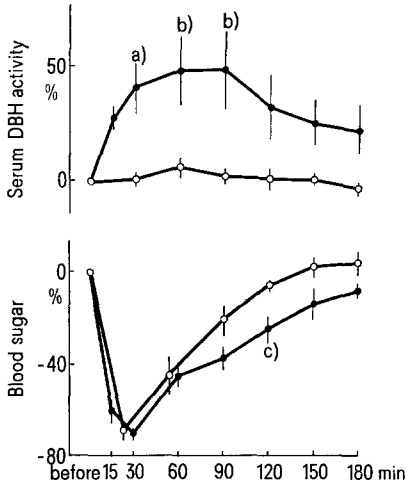
Elevation of Plasma Dopamine-β-Hydroxylase Activity during Insulin-Induced Hypoglycemia in Man

Dopamine-β-hydroxylase (DBH), the enzyme that converts dopamine to noradrenalin, is present in the synaptic vesicles of postganglionic sympathetic neurons[1,2]. The release of noradrenalin from the nerve terminal appears to occur via exocytosis. The soluble portion of DBH is also released simultaneously[3–5]. Therefore, it has been postulated that plasma DBH activity may serve as an index of the activity of the sympathetic nervous sytem[6,7]. This enzyme activity is increased in the blood of man under stresses such as cold pressor test and exercise[8]. But no significant change occurs during tilting[8]. In another report, physical exercise for 20 min on bicycle ergometer does not change the plasma activity, nor does electroshock treatment[9]. In the present study, we wish to determine changes of this enzyme activity during insulin-induced hypoglycemia, one of the strongest stresses known to increase sympathetic nerve activity[10].

The subjects consisted of 11 healthy medical students, all males, from 21 to 26 years of age. They were fasted in the morning and recumbent for 1 h prior to testing. Insulin was administered i.v. at 09.00 h in a single dose of 0.1 or 0.15 IU/kg body weight.

Blood samples for the determination of plasma DBH activity and blood sugar level were drawn immediately before the insulin and at intervals of 15–30 min for the next 3 h. Blood pressure and pulse rate were measured at the same time intervals. DBH activity was measured according to the procedure of NAGATSU and UDENFRIEND[11] and expressed as μmoles of octopamine formed per 1 l of plasma per 1 min of incubation at 37 °C.

In order to follow the catecholamine production, urine was collected during the control period: between 06.00 h and 09.00 h, and during the insulin test; 09.00 h and 12.00 h. The urinary adrenalin and noradrenalin were estimated according to the method of TAKAHASHI and GJESSING[12].



The changes of plasma DBH activity during insulin-induced hypoglycemia. Open circles, mean values obtained in 5 males administered with a dose of insulin 0.1 IU/kg body weight; solid circles, mean values obtained in 6 males administered with a dose of insulin 0.15 IU/kg body weight. DBH activity and blood sugar expressed in percent changes from their respective control values. [a] Significantly different ($p < 0.05$) from control. [b] Significantly different ($p < 0.01$) from control. [c] Significantly different between the value of a dose of 0.1 IU/kg and that of 0.15 IU/kg.

The Figure illustrates the responses of the mean plasma DBH activity and blood sugar level following insulin injection. DBH activity and blood sugar were represented in the percent changes from their respective control values. The mean blood sugar level fell markedly 30 min after insulin injection in both doses of 0.1 and 0.15 IU/kg. Then, gradual recoveries from hypoglycemia occurred toward the end of testings, the recovery after a dose of 0.15 IU/kg of insulin being slower than that after 0.1.

By the administration of 0.1 IU/kg of insulin, there was no significant increase of DBH activity, although a small peak was observed 60 min after injection. On the other hand, a striking elevation of this enzyme activity was seen by a dose of 0.15 from 30 min to 90 min after injection.

The mean values of the urinary catecholamines and circulatory variables are shown in the Table.

The adrenalin excretion for 3 h after the administration of insulin increased on an average 4.7 or 5.6 times with a dose of 0.1 or 0.15 IU/kg, respectively. The increases are highly significant as compared with the control values. But no significant difference was noted between the two dose levels, although the subjects given a larger dose of insulin showed more typical and marked symptoms of hypoglycemia.

There were no significant changes in the excretion of noradrenalin at either dosage level. However, this does not preclude the possibility that noradrenalin is actually released in larger quantity, since admittedly it is quickly taken up again at the sympathetic nerve endings, smooth muscle and glandular tissue[13] during stress, before being excreted in urine.

During a period of hypoglycemia, there was a significant increase in pulse rate at both dose levels, but blood pressure rose significantly only in subjects receiving a dose of 0.15 IU/kg.

It is well-known that the extra output of adrenalin in urine derives chiefly from the adrenals, and noradrenalin from the adrenergic nerves[10]. DBH is mainly present in the synaptic vesicles of post-ganglionic sympathetic neurons, and is released simultaneously with noradrenalin[1–5]. Therefore, the above findings, that urinary adrenalin and pulse rate increase significantly on administration of even a small amount of insulin, whereas plasma DBH level and systolic blood pressure rise only

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Plasma DBH activity, urinary catecholamine excretion, blood pressure and pulse rate before and during insulin-induced hypoglycemia

Insulin (U/kg)	Plasma DBH activity (μmoles/min/l plasma)		Adrenalin (μg/3 h)		Noradrenalin (μg/3 h)		Pulse rate (min)		Blood pressure (mm Hg)	
	Before insulin	After insulin[a]	Before insulin	After insulin	Before insulin	After insulin	Before insulin	After insulin[a]	Before insulin	After insulin[a]
0.1	22.4 ± 3.1[b] (5)	23.6 ± 2.8	2.7 ± 0.1	12.7 ± 1.7[e]	11.9 ± 1.5	10.9 ± 1.2	60.2 ± 3.2	72.4 ± 3.5[c]	129.2 ± 4.5	144.8 ± 5.7
0.15	16.8 ± 1.6 (6)	26.5 ± 1.3[e]	2.2 ± 0.3	12.3 ± 0.5[e]	10.2 ± 1.6	11.9 ± 1.7	53.3 ± 3.3	70.7 ± 5.8[c]	110.0 ± 1.2	137.0 ± 7.6[d]

[a] Highest level obtained during hypoglycemia. [b] Figures are given as mean ± standard error with number of specimens shown in parenthesis. The statistical significance of differences between the mean values of before and after insulin administration was certified by Student's *t*-test. [c] $p < 0.05$; [d] $p < 0.01$; [e] $p < 0.001$.

with a larger dose of insulin, may suggest that the adrenal glands react more promptly and extensively to a milder stress than do the sympathetic nerves.

The present data indicate a close correlation between the plasma DBH activity and the sympathetic nervous function during severe stress. The estimation of plasma DBH activity is highly useful in research and clinical practice to assess the sympathetic nervous activity, since it is of simpler procedure, less time-consuming and yields more stable and accurate values than the determination of plasma or urinary noradrenalin.

Résumé. Nous avons étudié l'activité de la dopamine-β-hydroxylase du plasma humain. Elle a été remarquablement accrue par l'administration de grandes quantités d'insuline. Les corrélations entre cette activité et celle des systèmes nerveux sympathiques sont également discutées.

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2 July 1974.

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Renal Blood Flow Changes During Aversive Conditioning in the Dog

In man and other animals, many aversive stimuli which elicit arterial hypertension also increase renal vascular resistance[1–3]. These and other observations[4] support the hypothesis that a causal relationship may exist between emotion-related renal vasoconstriction and hypertension. The frequency of occurrence of discrete aversive stimuli, under normal environmental conditions, might appear to be too low to account for sustained or frequent decreases in renal blood flow. An etiological mechanism for arterial hypertension might exist, however, if previously neutral, environmental cues became associated with aversive stimuli so that occurrence of either the aversive stimulus or the associated cues elicited renal vasoconstriction.

The results of the present study indicate that a previously neutral environmental cue, once associated with an aversive stimulus (using classical conditioning techniques) can, by itself, lead to neurally mediated renal vasoconstrictions.

Each of four adult, male mongrel dogs (12–16 kg) underwent several 1-hour habituation sessions in a restraint harness located within a specially-designed experimental chamber[5]. Then, during aseptic surgery, an electromagnetic flow transducer of appropriate diameter and an inflatable occlusion cuff (for determining zero blood flow) were fitted around the left renal artery. Care was taken to avoid injury to the renal nerves. In addition, an 18 gauge polyvinyl catheter was inserted into the aorta via a carotid artery. After recovery from surgery and stabilization of flow baselines (5 to 10 days) each dog was again placed in the chamber where continuous records of renal blood flow and arterial pressure were recorded.

The orienting reflex to the nonsystematic presentation of a 10-sec tone was habituated. Then each dog was exposed to a series of Pavlovian conditioning trials. A 10-sec tone (CS) was paired at termination with the occurrence of an unavoidable electric shock of 3–6 mA intensity and 0.5 sec duration (US) presented by a constant current source to the hind legs. 10 pairings were made during a 40 min period. Levels of mean arterial pressure, mean renal blood flow and renal vascular resistance were calculated from the polygraph tracings for each 2-sec interval beginning 6 sec before the CS onset and ending 10 sec after US onset.

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