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Preferential secretion of adrenaline or noradrenaline by the cat adrenal *in vivo* in response to different stimuli

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Summary

1. The concentrations of adrenaline and noradrenaline in the adrenal vein and the adrenal gland of the cat were studied in response to different stimuli leading to increased catecholamine (CA) secretion.

2. Haemorrhage and hypoglycaemia, but not acute exposure to cold or intravenous administration of cocaine, induced considerable increases in total catecholamine secretion.

3. The ratio of the concentration of adrenaline to noradrenaline in adrenal vein plasma during the control period was higher than the ratio in the adrenal gland itself.

4. Haemorrhage increased noradrenaline secretion considerably more than adrenaline secretion so that the ratio of the concentration of adrenaline to noradrenaline in adrenal vein plasma was significantly lower than in the adrenal gland itself.

5. Hypoglycaemia induced by insulin increased catecholamine secretion, with the adrenaline to noradrenaline ratio significantly higher than in the adrenal gland itself.

6. Hypothermia resulted in a fall of the initial high ratio of adrenaline to noradrenaline, to a value similar to that in the adrenal gland.

7. Neither cocaine nor changes in adrenal plasma flow affected the adrenaline to noradrenaline ratio in adrenal vein blood.

8. It is concluded that preferential release from the adrenal gland of either adrenaline or noradrenaline is possible *in vivo* in response to different stimuli.

Introduction

The chromaffin cells of the adrenal medulla of mammals contain adrenaline and noradrenaline (West, 1955). Histochemical studies with both the light and the electron microscope, suggested the presence of at least two types of chromaffin cells in the adrenal medulla: (1) noradrenaline-containing cells ; (2) adrenaline-containing cells (Benedeczky, Puppi, Tigyi & Lissak, 1965 ; Chang & Bencosme, 1968). Differential centrifugation yields two kinds of granules from the adrenal medulla: (a) large granules containing principally noradrenaline ; (b) small granules containing principally adrenaline (Yates, Wood & Duncan, 1962). The possible functional significance of the morphological differentiation of these catecholamines is unknown.

Thus, although electrical stimulation of the cat's hypothalamus altered the composition of the catecholamines secreted from the adrenal glands (Folkow & von Euler, 1954) and, in the dog, electrical stimulation of the splanchnic nerves innervating the adrenal gland (either intact or perfused) changed the composition of the catecholamines released from the gland (Mirkin, 1961 ; Vogt, 1965), no effective regulation of the amount of these amines was found in the dog by Lund (1951). Experiments on the perfused adrenal glands of cats have shown that pilocarpine, histamine, muscarine and polypeptides such as bradykinin, kallidin and angiotensin elicited a preferential release of adrenaline when added to the perfusion medium ; nicotine and KCl released principally noradrenaline (Rubin & Miele, 1968 ; Staszewska-Barczak & Vane, 1967). Rubin & Miele (1968) also showed that in the cat's perfused adrenal gland, different concentrations of KCl in the perfusion medium elicited release of different proportions of adrenaline and noradrenaline and, moreover, with the same perfusion medium, the catecholamine composition in the perfusate was found to be a function of the duration of the stimulation. Experiments with isolated bovine adrenal medullary granules have shown that Ca^{++}, ATP-magnesium and reserpine released principally noradrenaline, while tyramine preferentially released adrenaline (Oka, Ohuchi, Yoshida & Imaizumi, 1966). Bruinveils showed that *in vitro* incubation of cat adrenal slices with acetylcholine, increased preferentially the release of noradrenaline (Bruinveils, 1968).

The various investigations on *in vitro* preparations or perfused glands provide evidence in favour of the possibility that the adrenal medulla can release adrenaline or noradrenaline selectively but it is difficult to conclude from these data that such a differential release has a physiological significance, since most of the experiments were carried out under 'non-physiological' conditions. Furthermore, in some experiments the differentiation between adrenaline and noradrenaline was done by bioassay, which is not very specific. Thus, in order to evaluate the possible physiological role of preferential release of adrenaline or noradrenaline from the adrenal medulla it seemed desirable to use stimuli of a more 'physiological' nature and to test the response of the adrenal *in situ* with its natural perfusion of blood. Moreover, it seemed desirable to determine also the composition of the catecholamines in the gland itself. Vigorous stimuli may deplete the adrenal gland completely or partially but at the same time catecholamine synthesis continues. The composition of the catecholamines released under powerful stimulation of the gland may then reflect to some extent the rate of synthesis as depletion of the gland progresses. Therefore, in this investigation we have studied the release of catecholamines from the cat adrenal *in vivo*, induced by stress stimuli which did not result in appreciable depletion of the gland.

Methods

Cats of either sex weighing 2·2–5·8 kg were anaesthetized with pentobarbitone (40–50 mg/kg i.m.) and the right femoral vein was cannulated. In the experiments on bleeding and hypoglycaemia an additional polyethylene cannula was inserted into the right femoral artery. The cat was placed on its right side and the abdomen was opened 1–2 cm below the edge of the twelfth rib. The adrenolumbar vein was partially exposed and a ligature was placed at its junction with the inferior vena cava. Heparin (Pularin 1,000 U/kg) was injected intravenously, and a polyethylene cannula was inserted into the adrenolumbar vein and blood samples (6–9 ml) were

collected into ice-cooled test tubes containing 1·0 ml of 1·0% ascorbic acid and 1·0% disodium-EDTA solution. After withdrawal of each blood sample the volume was replaced by the injection of isotonic saline through the femoral vein. Stimuli were applied after two blood samples had been taken as control. All adrenolumbar vein samples were tested for catecholamines and no sample was discarded. The duration of the experiment was 60–80 min, unless stated otherwise.

Bleeding

(1) Two blood samples were taken as described, then 45–80 ml of blood were withdrawn at a rate of $7{\cdot}6 \pm 0{\cdot}9$ ml/min ; four–six additional samples of adrenal venous blood were collected. (2) Experiments were carried out as described in (1), but the average rate of bleeding was $3{\cdot}5 \pm 0{\cdot}7$ ml/minute. The difference between the two bleeding rates was significant ($P<0{\cdot}001$). In two experiments arterial blood was taken at the end of the experiment for determination of plasma catecholamines.

Hypoglycaemia

After two control blood samples had been collected, hypoglycaemia was induced by insulin (Squibb, regular) 10–15 units, injected intravenously. Serial blood samples were then collected, starting 30 min after the injection of insulin. A sample of 0·5–1·0 ml of arterial blood was taken simultaneously with each adrenal venous blood sample, and glucose was assayed with glucose oxidase.

Hypothermia

Control blood samples were taken as described. The animal was then placed in the cold room at 4–7° C ; four to five blood samples were collected while the rectal temperature gradually decreased over a period of 125 minutes.

Cocaine

After the collection of two control samples cocaine hydrochloride (5 mg/kg) was injected via the femoral vein ; two successive blood samples were then collected over 30 minutes.

At the end of the experiments the cats were killed by injection of pentobarbitone. The adrenal glands were immediately excised, cleaned, weighed and homogenized in ice-cold 0·4 N $HClO_4$ with an all-glass homogenizer. Homogenates were kept for 30 min in ice, then centrifuged in the cold for 5 min at 4,000 r.p.m. Supernatants were stored at 4° C until assayed for catecholamines.

Treatment of blood samples

Plasma was separated from erythrocytes by centrifugation for 5 min at 3,000 r.p.m. and 4° C. 4 N $HClO_4$ (1/10 of plasma volume) was used to precipitate protein. The supernatant was collected after centrifugation for 5 min. at 5,000 r.p.m. and stored at 4° C.

Isolation of catecholamines by ion exchange resin

Preparation of columns

Dowex 50W-x4, 200–400 mesh was used to prepare a column 4 cm high and 0·5 cm in diameter. For activation of the column the following solutions were used successively: 15 ml 2 N NaOH containing 1% disodium EDTA ; 40 ml H_2O ; 20 ml 2 N HCl ; 50 ml H_2O ; 15 ml 0·1 M phosphate buffer pH 6·5 (with 0·1% disodium-EDTA).

Adsorption of catecholamines on the columns

The same procedure was applied both for blood samples and adrenal glands. The pH of the sample was adjusted to 5·5 with 5 N K_2CO_3 and $KClO_4$ was sedimented by centrifugation for 5 min at 4,000 r.p.m. Samples were poured on to the columns, which were then washed successively with 20 ml 0·1% disodium-EDTA, 15 ml 0·1 M phosphate buffer, pH 6·5, and 5 ml H_2O. Adrenaline and noradrenaline were eluted together with 10 ml 1 N HCl and stored at 4° C. Adrenaline and noradrenaline concentrations were determined by the trihydroxyindole method as described by Kahane & Vestergaard (1965), with the following modifications: oxidation at pH 6·5 was carried out in ice for 15 min ; irradiation was omitted, the samples were allowed to stand for 45 min before reading the fluorescence. Fluorescence was measured in an Aminco-Bowman Fluoro-Microphotometer, using as primary filters Corning Nos. 5970 and 3850 and as secondary filters Corning Nos. 9780 and 3385.

To ascertain whether the adrenaline ratio of total catecholamines in adrenal vein blood varied spontaneously over a period equivalent to that used in the experiments with different stimuli, a group of cats ($n=6$) was prepared as described previously and six consecutive adrenal vein blood samples were collected and analysed for adrenaline and noradrenaline. The adrenaline ratio of the total catecholamines in the consecutive samples was: 74·3 ± 7·8% ; 78·6 ± 4·8% ; 74·4 ± 6·0% ; 77·8 ± 7·7% ; 74·5 ± 5·0% ($n=6$). None of the samples varied significantly in adrenaline ratio from any of the other preceding or consequent samples. The rate of total catecholamine secretion during the same period, in the consecutive collections was: 58·0 ± 11·6 ; 49·2 ± 6·4 ; 75·1 ± 10·2 ; 53·0 ± 8·3 ; 53·1 ± 11·4 ; 65·2 ± 8·5 (ng/kg)/10 minutes. Thus, no spontaneous increase in secretion rate during the period of the experiment was observed.

Results

Control period

The blood samples collected during the first control period were compared with those during the second period in forty-one experiments. As seen in Table 1 no

TABLE 1. *Adrenal plasma flow and catecholamines in adrenal venous blood during control period*

Blood sample	Adrenaline (% of total catecholamines)	Catecholamine concentration (μg/l. plasma)	Plasma flow (ml/10 min)
First sample	79·8 ± 2·5	*42·5 ± 3·8	5·6 ± 0·6
Second sample	78·3 ± 2·8	*27·9 ± 4·5	5·7 ± 0·5

The results are given as mean ± S.E.M. (standard error of the mean). * $P<0·001$.

TABLE 2. *Effect of various stimuli on adrenal secretion of catecholamines*

	Adrenaline (% of total catecholamines)			Catecholamine secretion rate ((ng/kg)/10 min)			Catecholamine concentration (μg/l. plasma)			Adrenal plasma flow (ml/10 min)		
	C	E	P*	C	E	P*	C	E	P*	C	E	P*
Acute haemorrhage (n=8)	81·8±7·3	32·3±6·3	<0·001	60·6±12·8	672±180	<0·02	44·3±9·1	1418±426	<0·02	4·7±1·9	2·2±1·2	<0·01
Attenuated haemorrhage (n=7)	81·6±3·9	31·4±5·5	<0·01	52·8±7·6	167±34	<0·001	27·5±1·8	212±43	<0·001	7·7±0·9	3·4±0·6	<0·001
Hypoglycaemia (n=9)	82·5±5·5	68·0±4·6	n.s.	52·4±7·9	441±79	<0·01	34·5±2·1	240±18	<0·01	5·2±0·7	6·6±1·2	n.s.
Hypothermia (n=6)	69·9±3·6	40·4±7·2	<0·01	66·1±17·0	65·9±17·0	n.s.	38·1±11·1	129±36	<0·05	5·5±0·7	1·8±0·3	<0·01

Results expressed as mean ± S.E.M. C, control period; E, end of period after appropriate stimulus. * Statistical evaluation of significance was performed using Student's t test for paired observations, comparing in each animal the two adrenal vein blood samples; P values are for the differences between control (C) and experiment (E); n.s., when $P>0·05$.

difference was found in adrenal vein plasma flow or the ratio of adrenaline to noradrenaline between the two control samples. However, the total catecholamine concentration in the first period was significantly higher ($P<0{\cdot}001$) than in the second period. Since plasma flow remained the same, the catecholamine secretion rate was higher during the first control period. This initial increased rate of catecholamine secretion may have resulted from the surgical procedures, including adrenolumbar-vein cannulation. The total catecholamine contents of thirty adrenal glands was 158 ± 9 μg/gland (668 ± 61 μg/g gland) and $49{\cdot}7\% \pm 1{\cdot}2\%$ of the total catecholamines in the glands was adrenaline.

Haemorrhage

Fast rate of bleeding

In all the experiments a considerable increase (average 11-fold) in total catecholamine secretion was found after bleeding. This increase was found with a decrease in plasma flow through the adrenal glands to less than 50% of that during the control period. These changes are depicted in a typical experiment shown in Fig. 1. After the bleeding there was a sluggish rise in adrenaline concentrations, but

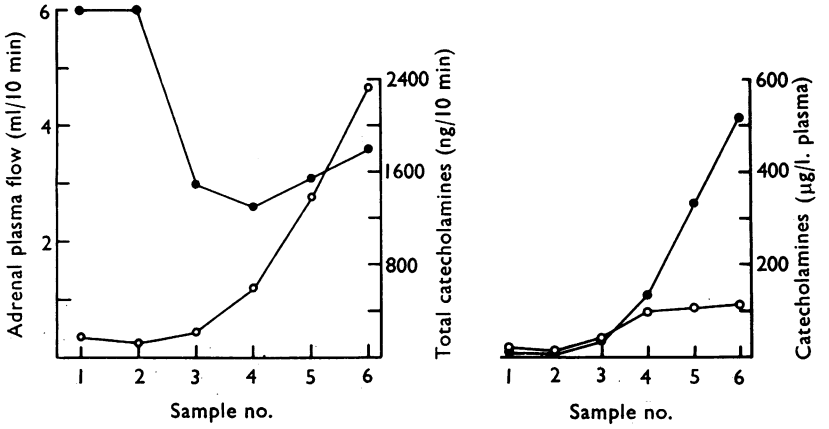

FIG. 1. Effect of fast bleeding on adrenal plasma flow and catecholamine secretion. Left plate: ○——○, total catecholamine secretion rate (ng/10 min) in each sample of adrenal venous blood; ●——●, plasma flow rate (ml/10 min). Right plate: ○——○, adrenaline concentration (μg/l. plasma); (●——●, noradrenaline concentration (μg/l. plasma) in adrenal venous blood.

TABLE 3. *Adrenal gland composition after various stimuli*

	Adrenaline (% of total catecholamines)	*P* (g-C)	*P* (g-E)
Acute haemorrhage ($n=8$)	$50{\cdot}9 \pm 3{\cdot}1$	$<0{\cdot}001$	$<0{\cdot}02$
Attenuated haemorrhage ($n=7$)	$52{\cdot}5 \pm 2{\cdot}4$	$<0{\cdot}001$	$<0{\cdot}01$
Hypoglycaemia ($n=9$)	$47{\cdot}3 \pm 1{\cdot}5$	$<0{\cdot}001$	$<0{\cdot}01$
Hypothermia ($n=6$)	$47{\cdot}6 \pm 3{\cdot}3$	$<0{\cdot}01$	n.s.

Results expressed as mean ± S.E.M. *P* (g-C) for difference between gland and control value of adrenal vein blood; *P* (g-E), for difference between gland and adrenal vein blood after appropriate stimulus.

a much more pronounced increase of noradrenaline so that the ratio of the concentration of adrenaline to noradrenaline decreased progressively, the lowest value being obtained in the sixth and last sample, at the end of the experiment. The mean of the values of the last samples of all the experiments is summarized in Table 2. In the adrenal gland itself adrenaline comprised about 51% of the total catecholamines (Table 3) which was significantly different from the composition of adrenal vein catecholamines both during the control period and after bleeding (see Table 3). The total amount of catecholamines secreted from the adrenal glands during the entire experiment was found to be 4·4% ±0·9% of the total catecholamines which were found in the adrenal glands at the end of the experiments. Thus it may indicate that there was no appreciable depletion of adrenaline or noradrenaline from the adrenal glands during the experiment.

Slow rate of bleeding

In this series of experiments the same type of response was found as that in the experiments using a fast rate of bleeding; however, the changes were more attenuated. The catecholamine secretion rate increased 3-fold as a result of the bleeding. Here again the percentage of adrenaline decreased significantly while that of noradrenaline increased, following bleeding. Adrenaline in the adrenal gland itself comprised a significantly lower percentage of total catecholamines than in the control blood samples and a significantly higher percentage than in adrenal vein plasma, after haemorrhage (Table 3). The total amount of catecholamines secreted from the adrenal gland throughout the experiment was 1·8% ±0·6% of the total catecholamines found in the adrenals at the end of the experiments, which is much less than during rapid bleeding. However, no significant difference in adrenal vein blood (percentage adrenaline) was found between the two series of haemorrhage experiments.

Hypoglycaemia

In eight out of nine experiments arterial blood was drawn for glucose determination. After insulin injection the concentration of plasma glucose decreased from

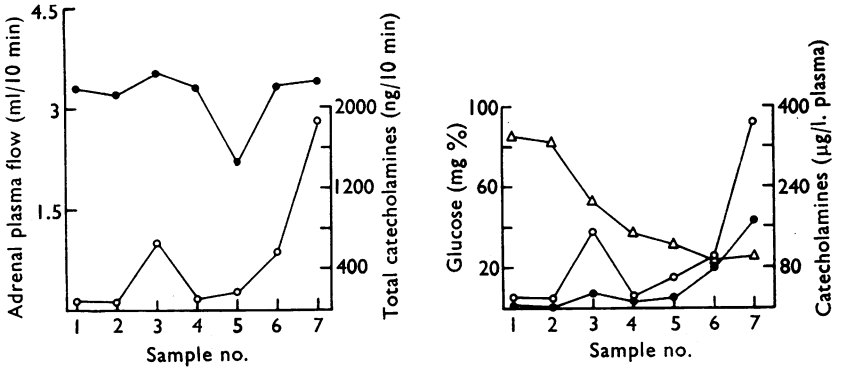

FIG. 2. Effect of insulin on blood glucose, adrenal plasma flow and catecholamine secretion. Left plate: ○——○, total catecholamines secretion rate (ng/10 min) in each sample of adrenal venous blood; ●——●, plasma flow (ml/10 min). Right plate: ○——○, adrenaline concentration (µg/l. plasma); ●——●, noradrenaline concentration (µg/l. plasma) in adrenal venous blood; △——△, glucose concentration in arterial blood (mg%).

$108 \pm 5{\cdot}0$ mg% in the control period to $44{\cdot}4 \pm 3{\cdot}8$ mg% at the end of the experiments ($P<0{\cdot}001$). Catecholamine secretion increased almost 9-fold simultaneously with the hypoglycaemia. Plasma flow through the adrenals did not change significantly throughout the experiment. The percentage of adrenaline in the adrenal vein in the hypoglycaemia experiments decreased somewhat but not significantly and remained higher than that in the adrenal gland itself (Table 3, $P<0{\cdot}01$). Figure 2 is a typical example of such an experiment where the first and the second samples are the control period and the 'end sample of the experiment' is the seventh one. The total amount of catecholamines secreted during the whole experiment was $2{\cdot}2\% \pm 0{\cdot}6\%$ of the total catecholamines in the glands at the end of the experiments.

Hypothermia

The average decrease of rectal temperature was $11{\cdot}0 \pm 1{\cdot}5°$ C over a period of 125 ± 14 minutes. In this type of experiment there was no increase of total catecholamine secretion rate while the temperature decreased, but the catecholamine concentration in adrenal vein blood increased more than 3-fold. This was due to a marked decrease in plasma flow to about one-third during hypothermia. At the end of the experiments adrenaline comprised $40{\cdot}4\% \pm 7{\cdot}2\%$ of total catecholamines in adrenal vein blood, which is significantly lower than during the control period (Table 2) but not significantly different from the composition of catecholamine in the adrenal glands (Table 3). The total catecholamines secreted from the adrenal glands during the whole experiment was $0{\cdot}9\% \pm 0{\cdot}1\%$ of the catecholamine content of the glands at the end of the experiment. These experiments show that a change in the ratio of adrenaline to noradrenaline may occur without any change in total catecholamine secretion rate.

Cocaine

After cocaine was injected, we found no significant increase of total catecholamine secretion rate in two consecutive adrenal vein blood samples. There was also no significant change of the ratio of adrenaline to noradrenaline in either the first or the second blood samples nor in the plasma flow through the adrenal gland (Table 4).

Discussion

The present investigation has shown that the ratio of adrenaline to noradrenaline released from the adrenal glands of a single species (cat) may be altered differently by several stress stimuli. In the anaesthetized 'undisturbed' cat adrenaline was initially approximately 80% of the total catecholamines in venous blood from the

TABLE 4. *Effect of cocaine on adrenal secretion of catecholamines*

	Adrenaline (% of total catecholamines)	Catecholamine secretion rate ((ng/kg)/10 min)	Plasma flow (ml/10 min)
Control	$79{\cdot}3 \pm 4{\cdot}3$	$56{\cdot}8 \pm 7{\cdot}3$	$5{\cdot}2 \pm 0{\cdot}8$
First blood sample after cocaine	$77{\cdot}0 \pm 4{\cdot}8$	$59{\cdot}5 \pm 9{\cdot}1$	$6{\cdot}6 \pm 1{\cdot}3$
Second blood sample after cocaine	$78{\cdot}9 \pm 4{\cdot}0$	$58{\cdot}3 \pm 8{\cdot}4$	$6{\cdot}0 \pm 1{\cdot}3$

The results are means of eleven experiments.

adrenals, while in the gland itself it accounted for only about 50% of total catecholamines. Therefore, the secretion 'at rest' was preferentially that of adrenaline. A similar finding has been observed in the dog, although the difference between the adrenal gland and the adrenal venous blood was less striking (Wurtman, Casper, Pohorecky & Bartter, 1968). Acute bleeding elicited an increased output of catecholamines which contained only about 30% of adrenaline, while hypoglycaemia (which was as powerful a stimulus as bleeding, as shown by the increased output of catecholamines) did not change significantly the ratio of adrenaline to noradrenaline. During hypothermia noradrenaline acounted for 60% of the total catecholamines released as compared to 30% during the control period. Notably the change of catecholamine composition in adrenal vein blood under hypothermia was not accompanied by any increase in the rate of secretion.

Only a small fraction of the total content of catecholamines of the adrenal gland was secreted during the entire period of the experiment. Thus, there was no significant depletion in the adrenal glands of either adrenaline or noradrenaline in response to any of the stimuli used in our experiments. Therefore, we may assume that the changes of the ratio of adrenaline to noradrenaline in adrenal vein blood were a consequence of preferential release of one or the other amine rather than changes in catecholamine composition within the glands.

Our results do not support, therefore, the conclusion of the review of Malmejac (1964) on adrenal medullary secretion that: 'There is no stress that causes obvious increase in the noradrenaline proportion of efferent adrenal blood. The relative proportion of adrenaline and noradrenaline remains generally constant or shifted in the direction of adrenaline secretion.'

The catecholamines released from nerve endings undergo reuptake to a considerable extent (Gillespie & Kirpekar, 1965 ; Hertting & Axelrod, 1961). Thus, it is possible that a different rate of reuptake of adrenaline or noradrenaline may change the composition of the initial secretion of the chromaffin cell. Moreover, the efficiency of the reuptake mechanism may change under different flow rates through the adrenals as Patton & Gillis (1965) showed for spleen, or as a result of the stimulated state of adrenergic neurones (Chang & Chiueh, 1968 ; Häggendal & Malmfors, 1969 ; Palaic & Panisset, 1969). These possibilities are based on some data accumulated over recent years: for example, an increase in noradrenaline reuptake by the perfused cat's spleen was found after electrical stimulation of the splenic nerve (Chang & Chiueh, 1968). On the other hand, electrical stimulation of the nerve to the vas deferens of the guinea-pig decreased noradrenaline uptake by that organ (Palaic & Panisset, 1969). Häggendal & Malmfors (1969) also showed a decrease in noradrenaline uptake by the iris and salivary glands of the rat after electrical stimulation of the nerves to these organs. In our experiments, the changes of catecholamine composition in the adrenal venous blood could not be correlated with the rate of secretion. (The rate of secretion may be taken as an expression of the stimulation of the chromaffin cells). Thus, in the experiments with fast and slow bleeding catecholamine secretion rate increased 11- and 3-fold, respectively, while the ratio of adrenaline to noradrenaline at the peak was the same in both types of haemorrhage. Hypoglycaemia elicited an output of catecholamines 8·5 times greater than during the control period while the ratio of adrenaline to noradrenaline ratio before and after induction of hypoglycaemia was unaltered. An increase in noradrenaline percentage without change in overall catecholamine secre-

tion rate was found during hypothermia. Comparison of the first control blood samples to the second ones (Table 1), revealed a decrease in catecholamine secretion rate but no change of the adrenaline to noradrenaline ratio. Thus, no correlation between rate of secretion and composition of catecholamines in adrenaline vein blood was found. The rate of plasma flow through the adrenals in these experiments was also unrelated to the ratio of adrenaline to noradrenaline.

A reuptake mechanism in the adrenal medulla may be altered by changes unrelated to the rate of secretion. In order to determine whether such a reuptake mechanism is of significance in the adrenal medulla we performed the experiments with cocaine, which is an effective inhibitor of the reuptake mechanism in sympathetic nerve endings (Haefely, Hurlimann & Thoenen, 1964; Whitby, Axelrod & Weil-Malherbe, 1961). But as seen in Table 4, no significant increase of catecholamine secretion rate was found after the injection of cocaine, nor was there any change of the ratio of adrenaline to noradrenaline. This would indicate that reuptake plays no significant role in the adrenal medulla. These findings corroborate various reports which have also indicated that cocaine does not affect uptake of catecholamines in the adrenal and some other tissues (Kirpekar & Cervoni, 1963; Fischer & Snyder, 1965).

The adrenal response to the different stress stimuli may be explained by the presence of two types of cells in the adrenal medulla: adrenaline cells and noradrenaline cells, which are under the control of different preganglionic nerve fibres, presumably originating in different 'centres' in the central nervous system. Since chromaffin cells secrete catecholamines in response to acetylcholine released from the preganglionic fibres, a homogeneous stimulation of the adrenal medulla would release catecholamines in a composition similar to their composition in the adrenal medulla, as suggested by the finding of Wurtman *et al.* (1968) that after hypophysectomy in the dog insulin caused secretion of catecholamines with a lower adrenaline proportion than controls, in parallel with the fall in adrenaline percentage in the adrenal gland after hypophysectomy. However, even in the hypophysectomized dogs insulin induced secretion of a somewhat higher proportion of adrenaline than in the gland itself.

In our experiments only a quantitative difference was found between fast and slow rates of bleeding. However, experiments on haemorrhage in dogs have shown that fast bleeding may alter the ratio of adrenaline to noradrenaline in favour of adrenaline while slow bleeding did not change the ratio of adrenaline to noradrenaline, though an increase in total catecholamine secretion was observed in both experiments (Walker, Zieleli, Reutter, Shoemaker, Friend & Moore, 1959). The difference between the observations of Walker *et al.* and our own report may be due to the different species used.

Hypothermia did not change the rate of total catecholamine secretion from the adrenal glands but did affect the adrenaline to noradrenaline ratio in favour of noradrenaline. Thus, hypothermia induced an increase in noradrenaline secretion together with a decrease in adrenaline secretion. The surprising lack of increased secretion of catecholamine from the adrenal under hypothermia may have been due to the relatively short duration of the experiment or to decreased responsiveness of the adrenal medulla under barbiturate anaesthesia. However, the other types of stimuli, for example, bleeding and hypoglycaemia, did elicit significant increases of

catecholamine output from the adrenal glands under the same conditions of anaesthesia.

The preferential release of noradrenaline in haemorrhage and of adrenaline in hypoglycaemia may be of functional importance. Thus, in haemorrhage vasoconstriction is an appropriate response and noradrenaline is more effective in this respect while in hypoglycaemia an increase in blood glucose is an appropriate response and adrenaline is the effective catecholamine in this respect. Although there was some fall in adrenaline proportion during hypoglycaemia, this fall was not statistically significant while the absolute concentration of adrenaline increased considerably and significantly.

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REFERENCES

BENEDECZKY, I., PUPPI, A., TIGYI, A. & LISSAK, K. (1965). Histochemical and electron microscopical study of the adrenal medulla of the grass snake (*Natrix natrix*). *Nature, Lond.*, **209**, 592–594.

BRUINVEILS, J. (1968). The influence of acetylcholine on the release of noradrenaline and adrenaline during incubation *in vitro* of adrenal slices from different species. *Archs Int. Pharmacodyn. Ther.*, **757**, 17–27.

CHANG, C. C. & CHIUEH, C. C. (1968). Increased uptake of noradrenaline in the rat submaxillary gland during sympathetic nerve stimulation. *J. Pharm. Pharmac.*, **20**, 157–159.

CHANG, W. W. L. & BENCOSME, S. A. (1968). Selective staining of secretory granules of adrenal medullary cells by silver methamine: A light and electron microscopic study. *Canad. J. Physiol. Pharmac.*, **46**, 745–747.

FISCHER, J. E. & SNYDER, S. H. (1965). Disposition of norepinephrine-H^3 in sympathetic ganglia. *J. Pharmac. exp. Ther.*, **150**, 190–195.

FOLKOW, B. & VON EULER, U. S. (1954). Selective activation of noradrenaline and adrenaline producing cells in the adrenal gland by hypothalamic stimulation. *Circulation Res.*, **2**, 191–195.

GILLESPIE, J. S. & KIRPEKAR, S. M. (1965). Uptake and release of H^3-noradrenaline by the splenic nerves. *J. Physiol., Lond.*, **178**, 44–47.

HAEFELY, W., HURLIMANN, A. & THOENEN, H. (1964). A quantitative study of the effect of cocaine on the response of the cat nictitating membrane to nerve stimulation and to injected noradrenaline. *Br. J. Pharmac. Chemother.*, **22**, 5–21.

HÄGGENDAL, J. & MALMFORS, T. (1969). The effect of nerve stimulation on the uptake of noradrenaline into the adrenergic nerve terminals. *Acta physiol. scand.*, **75**, 28–32.

HERTTING, G. & AXELROD, J. (1961). Fate of tritiated noradrenaline at the sympathetic nerve endings. *Nature, Lond.*, **192**, 172–173.

KAHANE, Z. & VESTERGAARD, P. (1965). An improved method for measurement of free epinephrine and norepinephrine with a phosphate-metaphosphate buffer in the trihydroxyindole procedure. *J. lab. clin. Med.*, **65**, 848–858.

KIRPEKAR, S. M. & CERVONI, P. (1963). Effect of cocaine, phenoxy-benzamine and phentolamine on the catecholamine output from spleen and adrenal medulla. *J. Pharmac. exp. Ther.*, **142**, 59–70.

LUND, A. (1951). Release of adrenaline and noradrenaline from the suprarenal gland. *Acta pharmac. tox.*, **7**, 309–320.

MALMEJAC, J. (1964). Activity of the adrenal medulla and its regulation. *Physiol. Rev.*, **44**, 186–218.

MIRKIN, B. L. (1961). Factors influencing the selective secretion of adrenal medullary hormones. *J. Pharmac. exp. Ther.*, **132**, 218–225.

OKA, M., OHUCHI, T., YOSHIDA, H. & IMAIZUMI, R. (1966). Selective release of noradrenaline and adrenaline from isolated adrenal medullary granules. *Life Sci.*, **5**, 433–438.

PALAIC, D. & PANISSET, J. C. (1969). Inhibition of the noradrenaline uptake in guinea-pig vas deferens by continuous nerve stimulation. *J. Pharm. Pharmac.*, **21**, 328–329.

PATTON, D. M. & GILLIS, C. N. (1965). Effect of altered perfusion rates on the retention of noradrenaline by the spleen. *Nature, Lond.*, **208**, 391–392.

RUBIN, R. P. & MIELE, E. (1968). A study of the differential secretion of epinephrine from the perfused cat adrenal gland. *J. Pharmac. exp. Ther.*, **164**, 115–121.

STASZEWSKA-BARCZAK, J. & VANE, J. R. (1967). The release of catecholamines from the adrenal medulla by peptides. *Br. J. Pharmac. Chemother.*, **30**, 655–667.

VOGT, M. (1965). Release of medullary amines from the isolated perfused adrenal gland of the dog. *Br. J. Pharmac. Chemother.*, **24**, 561–565.

WALKER, W. F., ZIELELI, M. S., REUTTER, F. W., SHOEMAKER, W. C., FRIEND, O. & MOORE, E. O. (1959). Adrenal medullary secretion in haemorrhagic shock. *Am. J. Physiol.*, **197**, 773–780.

WEST, G. B. (1955). The comparative pharmacology of the suprarenal medulla. *Q. Rev. Biol.*, **30**, 116–137.

WHITBY, L. G., AXELROD, J. & WEIL-MALHERBE, H. (1961). The fate of H^3-norepinephrine in animals. *J. Pharmac. exp. Ther.*, **132**, 193–197.

WURTMAN, R. J., CASPER, A., POHORECKY, L. A. & BARTTER, F. C. (1968). Impaired secretion of epinephrine in response to insulin among hypophysectomized dogs. *Proc. Natn. Acad. Sci., U.S.A.*, **61**, 522–528.

YATES, D. H., WOOD, J. G. & DUNCAN, D. (1962). Phase and electron microscopic observations on two cell types in the adrenal medulla of the Syrian hamster. *Texas Rep. Biol. Med.*, **20**, 494–511.

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