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EXOCYTOTIC RELEASE OF CATECHOLAMINE FROM PERFUSED ADRENAL GLAND OF GUINEA-PIG INDUCED BY VERATRIDINE

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1 Experiments were carried out on perfused adrenal glands of guinea-pig to determine whether veratridine caused the exocytotic release of catecholamine by comparing its effect with that of splanchnic nerve stimulation and secretagogues such as acetylcholine and excess K^+.

2 Veratridine (100 μM) and excess K^+ (56 mM) caused secretion of catecholamine and dopamine-β-hydroxylase (DBH) activity in the venous effluents in the presence of atropine (30 μM) and hexamethonium (2 mM). Splanchnic nerve stimulation in the presence or absence of physostigmine (100 nM) and infusion of acetylcholine in the presence of physostigmine had the same effect. In all the responses, the release of DBH tended to last for a longer period than that of catecholamine.

3 The ratio of catecholamine to DBH activity appearing in the venous effluents was approximately 9, regardless of the method of stimulation. This value was close to the ratio of catecholamines to the 'soluble' DBH activity found in the chromaffin granules.

4 All the types of stimulation used caused a proportional release of adenine nucleotides and catecholamines in the effluents. The adenine nucleotides were mainly adenosine 5'-phosphate.

5 The ratio of catecholamine to adenine nucleotides was approximately 11, regardless of the method of stimulation.

6 It is suggested that the release of catecholamine induced by veratridine occurs by exocytosis in adrenal glands of guinea-pig.

Introduction

Veratridine causes a tetrodotoxin-sensitive increase in catecholamine output from perfused adrenal glands of guinea-pig, suggesting that adrenal chromaffin cell membranes possess Na^+ channels responsible for the catecholamine secretion under normal conditions (Ito, Nakazato & Ohga, 1978; 1979). It is uncertain whether the release of catecholamines induced by veratridine occurs by exocytosis. The biochemical evidence for the exocytotic release of catecholamines from adrenal chromaffin cells is that the 'soluble' contents of granules such as adenosine 5'-triphosphate (ATP) (Douglas, Poisner & Rubin, 1965; Banks, 1966; Douglas & Poisner, 1966a,b; Lastowecka & Trifaró, 1974), chromogranin A (Banks & Helle, 1965; Blaschko, Comline, Schneider, Silver & Smith, 1967; Kirshner, Sage & Smith, 1967; Schneider, Smith & Winkler, 1967) and dopamine-β-hydroxylase (DBH) (Viveros, Arqueros & Kirshner, 1968; Sorimachi & Yoshida, 1979) are released with catecholamine in the same proportion as they are found in the lysate of the isolated chromaffin granules. In the present experiments, we tried to discover whether the release of catecholamines induced by veratridine was accompanied by release of DBH and ATP from perfused, isolated adrenal glands of guinea-pig. The results were compared with the effects of splanchnic nerve stimulation, acetylcholine (ACh) and excess K^+ which are reported to cause the secretion of DBH (Viveros *et al.*, 1968; Dixon, García & Kirpekar, 1975; Jacobs, Henry, Johnson & Williams, 1978; Sorimachi & Yoshida, 1979) or adenine nucleotides (Douglas *et al.*, 1965; Banks, 1966; Douglas & Poisner, 1966a) together with catecholamines in other species.

Methods

Perfusion of guinea-pig isolated adrenal glands

Guinea-pigs weighing 500 to 800 g were anaesthetized with sodium pentobarbitone (40 mg/kg) intraperitoneally. Both adrenal glands were perfused and isolated following the general procedure described previously (Ito *et al.*, 1978; 1979). If necessary, the adrenal glands were isolated together with right and left greater splanchnic nerves. Peripheral ends of the

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nerves were drawn into a glass suction electrode containing Locke solution and stimulated with supramaximal voltage of 1 ms duration at a frequency of 30 Hz for 10 min. The polarity of the electrode was changed every minute.

The standard perfusion medium was Locke solution of the following composition (mM); NaCl 154, KCl 5.6, $CaCl_2$ 2.2, Na_2HPO_4-NaH_2PO_4 buffer (pH 7.2) 3 and glucose 10. Pure O_2 was continuously bubbled through this solution and the perfusion was carried out at 24° ± 1°C. In solutions containing 56 mM K, the NaCl content was reduced correspondingly. Perfusion medium containing veratridine was prepared by the addition of an appropriate quantity of a stock solution of the drug dissolved in dimethylsulphoxide. The final concentration of this solvent was 0.1% which had no effect on catecholamine release. The required quantities of ACh were added to Locke solution containing physostigmine (100 nM) from concentrated stock solution. When excess K or veratridine was infused, all solutions contained atropine (30 μM) and hexamethonium (2 mM) to prevent indirect effects through the activation of both muscarinic and nicotinic receptors on the chromaffin cells. The infusion of drugs was started 30 to 60 min after the beginning of the perfusion.

Subcellular fractionation of adrenal glands

Highly purified chromaffin granules were obtained by the method of Smith & Winkler (1967) as modified by Dixon *et al.* (1975).

Assay of catecholamine

Samples of the subcellular fraction or perfusates were acidified with 8 N perchloric acid giving a final concentration of 0.4 N. The acidified sample was centrifuged at 25,000 *g* at 5°C for 10 min to remove proteins. The clear supernatant was transferred to a glass test tube and was stored on ice until assay which was carried out on the day of the experiment. Catecholamine (adrenaline) content of the sample was assayed by the fluorimetric method of Anton & Sayre (1962). Values of catecholamine are expressed in nmol.

Assay of dopamine-β-hydroxylase

Bovine serum albumin (2 mg) was added to each adrenal effluent (3 or 3.5 ml) which was then dialyzed with ammonium sulphate solution (80%) containing K phosphate buffer (pH 6.5) 20 mM for 15 h. After centrifugation (25,000 *g* for 10 min), the precipitates were dissolved in cold distilled water (1.3 to 2 ml). DBH activity of 0.8 ml of the sample was assayed. The percentage of recovery for dialysis was from 72 to 88% and data were not corrected for recovery. To estimate 'soluble' and 'membrane bound' DBH activity, 0.2 ml of the sample without dialysis was assayed.

DBH activity was measured by a modification of the methods of Friedman & Kaufman (1965) using uniformly labelled [^{3}H]-tyramine as a substrate. One ml of the reaction mixture containing K-phosphate buffer (pH 5.5) 100 mM, tyramine (3 μCi) 9.2 μM, fumarate 50 mM, tranylcypromine 0.5 mM, ascorbate 1 mM and catalase, 1,500 units was incubated for 1 h at 37°C in air. The reaction was stopped with 1 ml of 6% metaphosphoric acid. After removing the precipitate, the supernatant was used to determine the amount of octopamine formed by oxidation with periodate as described by Friedman & Kaufman (1965).

The partially purified bovine adrenal DBH was used as a standard, the activity of which was also checked spectrophotometrically using non-labelled tyramine as a substrate (Kato, Kuzuya & Nagatsu, 1974). Linear correlation was obtained between absorbance and ct/min and thus ct/min was converted to units of DBH activity. DBH activity was expressed as nmol/h octopamine formed from tyramine. Locke solution containing bovine serum albumin (2 mg) was dialyzed and served as blank. This blank value was not different from that obtained from the boiled sample of 'soluble' DBH.

When the subcellular fraction was assayed, various concentrations of $CuSO_4$ (2 to 10 μM) were added to the reaction mixtures in order to prevent the activity of the endogenous inhibitors of DBH. Maximum DBH activity obtained in the presence of Cu^{2+} ions was expressed as a 'soluble' or 'membrane bound' DBH activity. Endogenous inhibitors in the adrenal effluents were checked by adding a known amount of partially purified bovine adrenal DBH to a duplicate of each sample. The decrease of bovine DBH activity was expressed as activity of endogenous inhibitors (Orcutt & Molinoff, 1977).

Assay of adenine nucleotides

ATP was measured by the luciferine-luciferase method originally described by Strehler (1963). Adenosine 5'-pyrophosphate (ADP) and AMP were determined after enzymatic conversion to ATP by the method of Imai, Riley & Berne (1964) as modified by Douglas & Poisner (1966a). Firefly tail powder was purified by a modification of the method of Nielsen & Rasmussen (1968).

The venous effluent was acidified with 8 N perchloric acid (giving a final concentration of 0.4 N) and 0.3 ml of triethanolamine (1 M) was added to this acidified effluent (3 ml). The pH of the sample was adjusted to 7.4 by gradual addition of K_2CO_3 (5 M) and the sample was left to stand on ice for 1 h. The precipitate was removed with filter paper and the

clear sample was frozen until assay. By this procedure, adenine nucleotides were fully recovered.

ATP was measured as follows: 0.4 ml of sample or standard solution was placed in a cuvette set in front of the photomultiplier. The luciferine-luciferase solution (0.7 ml) was rapidly injected into the cuvette through a polyethylene tube with a 1 ml glass syringe. The syringe was driven by constant air pressure to keep the injection time constant. Output from the amplifier was recorded with a pen recorder. The peak deflection of the pen was linearly related to the concentration of ATP, ADP and AMP in the standard solution. The conversion ratio of ADP to ATP and that of AMP to ATP was more than 95 and 80%, respectively. Chloride ions have some quenching effect on the luciferine-luciferase reaction (Strehler, 1963). Therefore, a standard solution for determining the amount of adenine nucleotides in the sample was prepared by adding known amounts of ATP, ADP and AMP to Locke solution treated with perchloric acid, triethanolamine and K_2CO_3 in the same way as described above.

Adrenal effluents usually contained small amounts of blood which was a source of adenine nucleotides. The ATP, ADP and AMP concentrations in guinea-pig blood diluted 100 parts/10^6 were 25, 4 and 4 pmol/ml, respectively. Therefore, the amount of blood in the venous effluents was assayed by benzidine reagents (Bing & Baker, 1931).

Results

Release of catecholamine and dopamine-β-hydroxylase induced by splanchnic nerve stimulation, acetylcholine, excess K and veratridine

After 20 to 50 min of initial perfusion of the glands with Locke solution, secretion of catecholamines and DBH activity was low, usually less than 1 nmol and 0.1 nmol/h per 10 min collection period, respectively. Electrical stimulation of the splanchnic nerve and infusion of acetylcholine (ACh, 30 μM) for 10 min caused an increase in the release of both catecholamine and DBH activity in the venous effluents in the presence of physostigmine (100 nM) (Figure 1a). The catecholamine output induced by such simulation returned to near the resting value 10 to 20 min after the stimulation period. The release of DBH reached the highest value during the period of stimulation, but fell gradually taking over 50 min or more. Splanchnic nerve stimulation sometimes caused a further increase in the catecholamine output even after the termination of stimulation (Figure 2). In this case, DBH activity in the effluents developed gradually and reached the maximum value lagging behind the maximum of catecholamine output.

Infusion of excess K (56 mM) and veratridine (100 μM) for 10 min also increased the release of catecholamines and DBH in the presence of atropine (30 μM) and hexamethonium (2 mM) (Figure 1b). As already reported (Itō *et al.*, 1978; 1979), the catecholamine output induced by excess K returned almost to the resting value during the 10 min post-stimulation period but that caused by veratridine developed gradually and decreased more slowly. The DBH secretion induced by excess K attained a maximum during the period of stimulation and then decreased gradually in the same way as that obtained by splanchnic nerve stimulation and by infusion of ACh. On the other hand, the time course of DBH secretion induced by veratridine was slower than that of catecholamines and the maximum value of DBH activity lagged behind that of catecholamines. The DBH activity decreased to near the resting value, taking 70 min or more.

Difference in the time course of release of catecholamine and dopamine β-hydroxylate activity

As indicated above, all the methods of stimulation, including veratridine, were effective in releasing DBH activity with catecholamie from guinea-pig perfused adrenal glands. Since the time course of release of DBH activity was slower than that of catecholamines, possible reasons for this were examined. First, it was determined whether or not the venous effluents contained endogenous inhibitors which modify DBH activity. To test this, a known amount of partially purified bovine adrenal DBH was added to a duplicate of each sample (Orcutt & Molinoff, 1977). As shown in Figure 1(a) and (b) the activity of DBH added exogenously was not affected throughout the experiments except that it decreased to 75 and 85%, when higher DBH secretion was obtained by infusion of excess K^+ and veratridine, respectively. These rates of inhibition are not enough to change the whole time course of DBH secretion. Secondly, in order to ascertain whether there was any loss in DBH activity during its passage through the glands, bovine adrenal DBH was infused into the artery for 10 min and the enzyme activity was measured in the venous effluents. As shown in Figure 3b, most of the DBH activity was recovered in the effluents without any delay, suggesting that DBH is not retained in the adrenal circulation during its passage. Thirdly, it might be possible that uptake of catecholamine is responsible for a more rapid disappearance of catecholamine from the effluents than DBH. This was tested by comparing the response induced by excess K in the presence and absence of phenoxybenzamine which blocks the uptake mechanism in peripheral sympathetic nerves. Figure 3a shows that the time course of catecholamine output was not affected by the presence of

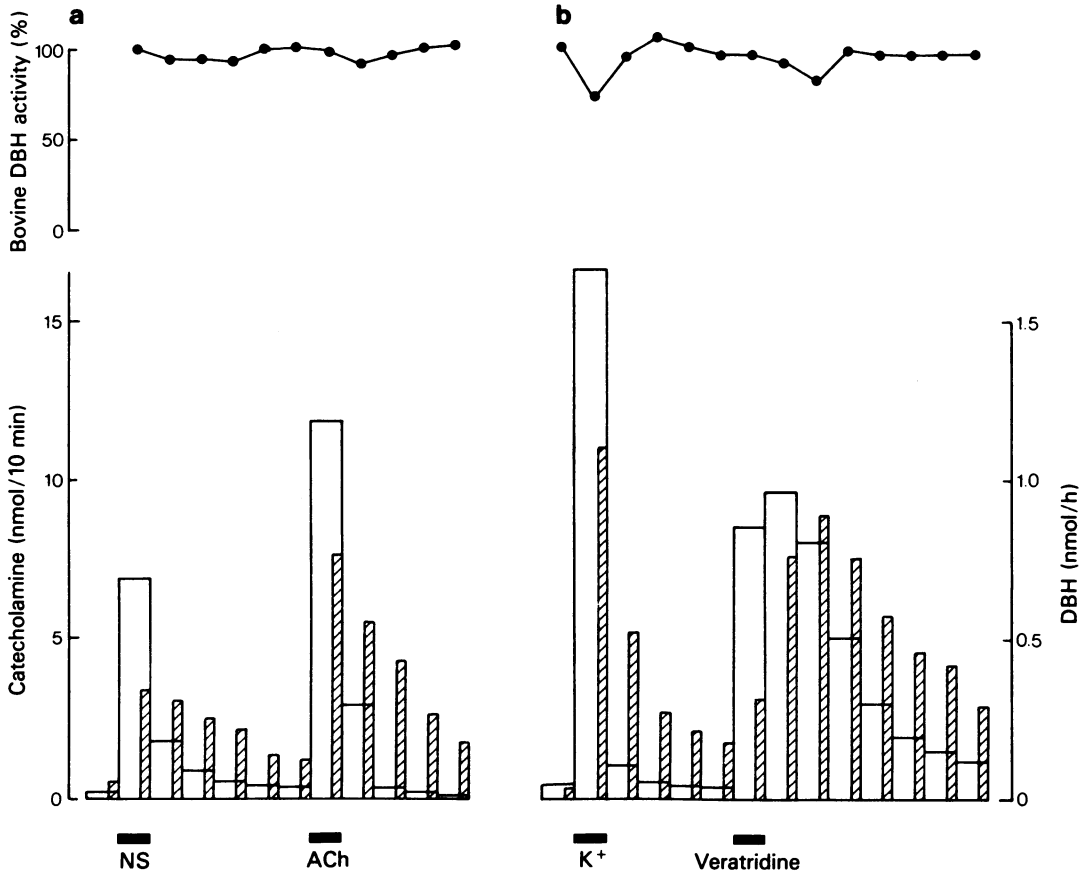

Figure 1 Release of catecholamine and dopamine-β-hydroxylase (DBH) from pefused guinea-pig adrenal glands induced by splanchnic nerve stimulation and acetylcholine in the presence of physostigmine (100 nM) (a) and by excess K^+ and veratridine in the presence of atropine (30 μM) and hexamethonium (2 mM) (b). Columns represent the amounts of catecholamine (open) and DBH activity (hatched) in the venous effluent collected for each 10 min. Filled horizontal bars indicate the period of splanchnic nerve stimulation (NS, 30 Hz, 1 ms, supramaximum voltage), infusion of acetylcholine (ACh, 30 μM), excess K^+ (K^+, 56 mM) and veratridine (100 μM) for 10 min. Upper traces indicate changes in the activity of endogenous inhibitors of DBH during experiments. Bovine adrenal DBH (0.39 nmol/h in (a) and 0.33 nmol/h in (b)) was added to a duplicate of each sample collected for 10 min and the percentage change in their activity in each 10 min collection was plotted (●).

phenoxybenzamine (10 μM), suggesting that the rapid disappearance of catecholamine in the effluents does not result from the uptake of catecholamine.

Ratio of catecholamine to dopamine β-hydroxylase in chromaffin granules and in adrenal effluents

Chromaffin granules Evidence for exocytotic release of catecholamines in adrenal chromaffin cells is that the ratio of catecholamine to DBH in the effluents is close to that found in chromaffin granules (Viveros *et al.*, 1968; Lastowecka & Trifaró, 1974; Sorimachi & Yoshida, 1979). Thus the ratio of catecholamine to 'soluble' DBH in the chromaffin granules was estimated using highly purified chromaffin granules which were obtained by the discontinuous sucrose density gradient technique (Smith & Winkler, 1967). Osmotic lysis of these granules solubilized 63 ± 2% ($n = 6$) of the total DBH activity together with most of the catecholamine in the granular fraction. This value was similar to that (71 ± 4%) found by Arnaiz, García, Horga & Kirpekar (1978). The ratio of catecholamine to 'soluble' DBH activity ranged from 6.86 to 11.1 (8.92 ± 0.55) in six experiments.

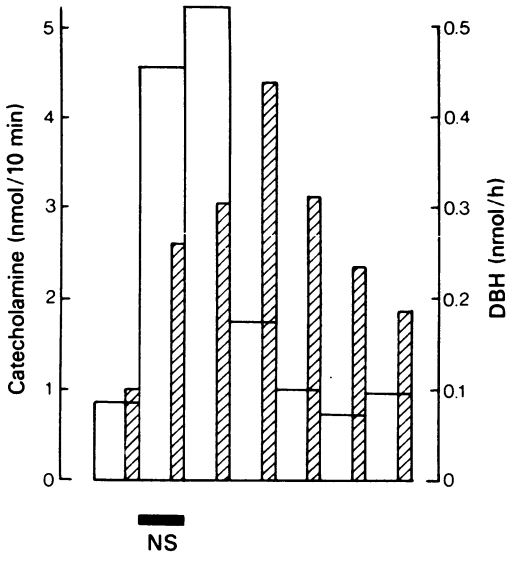

Figure 2 A different type of release from guinea-pig adrenal glands of catecholamine and dopamine-β-hydroxylase induced by splanchnic nerve stimulation in the absence of physostigmine. For further explanation see legend to Figure 1.

Evoked release Since the time course of DBH and catecholamine secretion in the venous effluents was somewhat different, the ratio of catecholamine to DBH was variable in each collection period. Therefore the total amounts of catecholamine and DBH released 50, 40, 40 and 70 min during and after splanchnic nerve stimulation, infusion of ACh, excess K and veratridine were compared. The results were summarized in Table 1. The ratio of catecholamine to DBH activity varied from 7.57 to 14.34 but there was no significant difference between the mean values (splanchnic nerve stimulation, 8.7 ± 0.51; ACh, 8.56 ± 0.29; excess K, 10.22 ± 0.80; veratridine, 8.66 ± 0.32, mean $\pm$ s.e.) with different methods of stimulation and that found in chromaffin granules (8.92 ± 0.55).

Release of adenine nucleotides induced by splanchnic nerve stimulation acetylcholine, excess K and veratridine

Secretion of catecholamine is accompanied by a simultaneous release of AMP and its metabolites from perfused cat (Douglas *et al.*, 1965; Douglas & Poisner, 1966a,b) and bovine (Banks, 1966) adrenal gland. In the previous section, the time course of release of

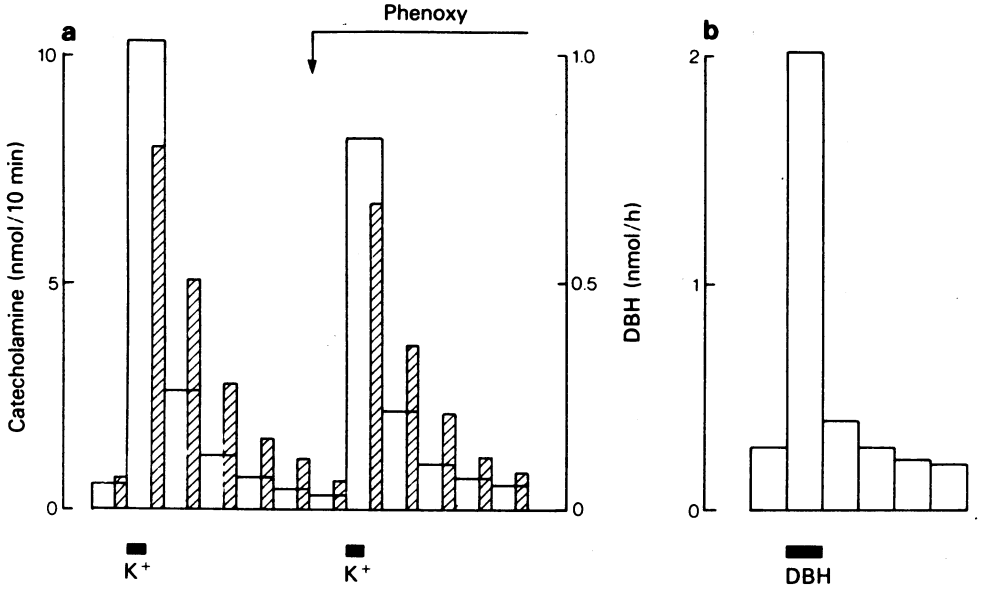

Figure 3 Release of catecholamine and dopamine-β-hydroxylase (DBH) induced by excess K^+ in the presence and absence of phenoxybenzamine (10 μM) (a) and recovery of bovine adrenal DBH from venous effluents (b). In (a), columns represent the amounts of catecholamines (open) and DBH activity (hatched) in the effluents collected every 10 min. In (b), columns show DBH activity in the effluents collected for 10 min. Filled horizontal bars indicate the period of infusion of excess K^+ (56 mM) for 5 min (a) and bovine adrenal DBH (2 nmol/h) for 10 min (b). Phenoxybenzamine (Phenoxy 10 μM) was present for the period indicated by a horizontal bar starting at the arrow.

Table 1 Ratios of catecholamine (CA) to dopamine-β-hydroxylase (DBH) in the perfusates from guinea-pig adrenal glands

Nerve stimulation (50 min)			*Acetylcholine* (40 min)			*Excess K* (40 min)			*Veratridine* (70 min)		
No.	CA	CA/DBH	No.	CA	CA/DBH	No.	CA	CA/DBH	No.	CA	CA/DBH
1.	10.56	8.52	1.	15.32	7.74	2.	33.78	11.50	2	76.54	8.86
4.	14.45	9.26	6.	21.02	8.80	3.	18.66	8.84	3	37.81	8.90
4.	10.29	7.57	6.	13.50	7.80	8.	14.98	8.51	13.	34.82	7.97
5.	6.41	8.01	7.	13.67	8.65	8.	12.11	8.76	14.	47.00	9.63
5.	4.02	7.88	7.	9.84	9.65	9.	36.27	14.34	15.	30.63	7.94
10.	21.73	10.97	11.	27.71	8.69	9.	22.59	9.15			
						12.	23.25	10.43			
Mean ± s.e.		8.70 ± 0.51			8.56 ± 0.29			10.22 ± 0.80			8.66 ± 0.32

Total amounts of catecholamine (nmol) (CA) and ratios of catecholamine (nmol) to DBH activity (nmol/h) (CA/DBH) during and after splanchnic nerve stimulation (30 Hz, 1 ms, supramaximum voltage), infusion of acetylcholine (30 μM), excess K (56 mM) and veratridine (100 μM) for 50, 40, 40 and 70 min, respectively, are shown. The same experimental numbers (No.) indicate results obtained from the same perfused adrenal glands.

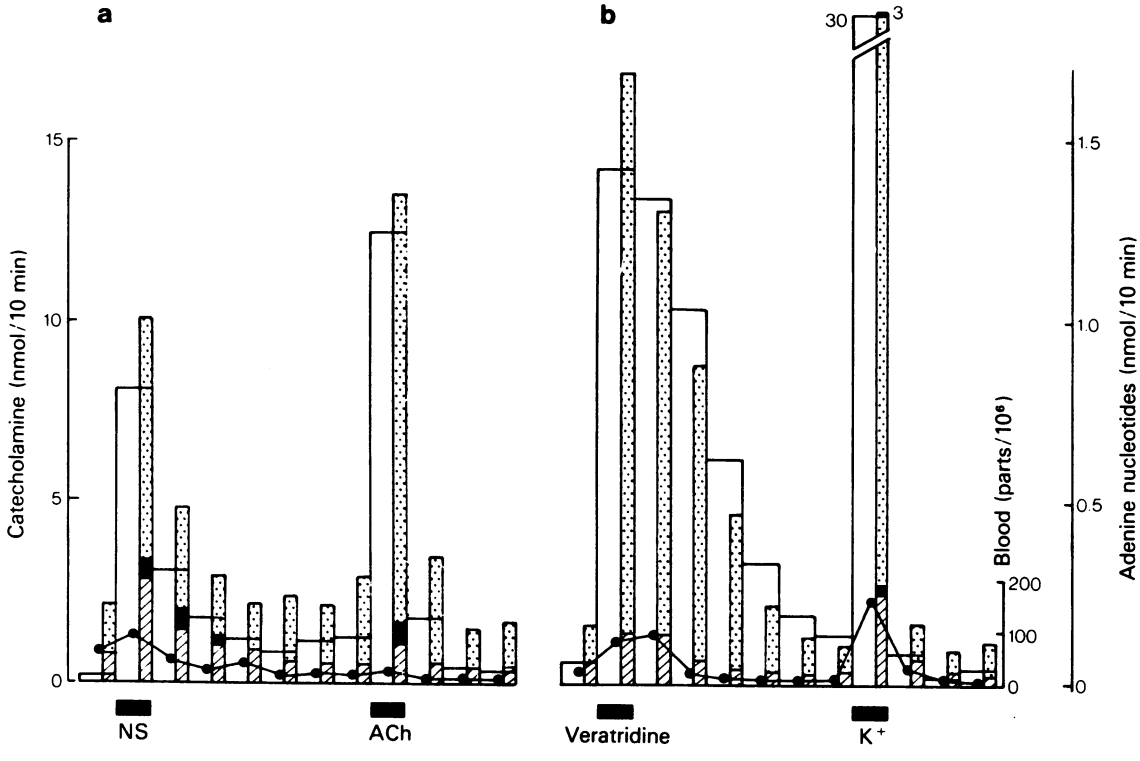

Figure 4 Release of catecholamine and adenine nucleotides induced by splanchnic nerve stimulation and acetylcholine in the presence of physostigmine (100 nM) (a) and by excess K^+ and veratridine in the presence of atropine (30 μM) and hexamethonium (2 mM) (b). Columns represent the amounts of catecholamine (open) and adenine nucleotides (AMP, strippled; ADP, filled; ATP, hatched) in the effluent collected every 10 min. Filled circles indicate both the amounts of blood and adenine nucleotides originating from blood. Filled horizontal bars indicate the periods of splanchnic nerve stimulation (NS, 30 Hz, 1 ms, supramaximum voltage) and infusion of acetylcholine (ACh, 30 μM), veratridine (100 μM) and excess K^+ (K^+, 56 mM) for 10 min, respectively.

catecholamine and DBH was found to be different. Therefore, it was of interest to determine whether ATP is released in the venous effluents with a similar time course to that of catecholamines in response to the different types of stimulation.

Figure 4a shows a typical result of the release of catecholamine and adenine nucleotides induced by splanchnic nerve stimulation and by infusion of ACh (30 μM) in the presence of physostigmine (100 nM). Both types of stimulation caused a parallel increase and decrease in the release of catecholamine and adenine nucleotides in the venous effluents. Similar results were also obtained in response to veratridine and excess K in the presence of atropine and hexamethonium (Figure 4b).

It should be noted that small amounts of blood present in the effluents, could be a source of adenine nucleotides (see Methods),. The resting content of blood found in the effluents during an initial 10 min collection period varied from gland to gland (4 to 61 parts/10^6 blood, 31 ± 6 parts/10^6, mean ± s.e., $n = 8$). Although blood in the effluents decreased with time of perfusion, it increased in response to stimulation (Figure 4a and b). Therefore, the amounts of ATP, ADP and AMP originating from blood were subtracted from those found in the adrenal effluents and the ratio of ATP, ADP and AMP to the total adenine nucleotides in each collection period were estimated. The results indicate that the largest part of the adenine nucleotides was AMP (75 to 96%), most of the rest was ATP and amounts of ADP were very low throughout the experiments. The ratio of AMP appears to increase with the increase in the output of adenine nucleotides in response to splanchnic nerve stimulation and secretagogues.

Relationship between the evoked secretion of catecholamine and adenine nucleotides

In order to estimate the relationship between the evoked secretion of catecholamine and adenine nucleotides, the amounts of adenine nucleotides appearing in the venous effluent obtained during the 10 min collection period of splanchnic nerve stimulation or infusion of secretagogues, were plotted against amounts of catecholamine. With splanchnic nerve stimulation and veratridine, the plots also show values for 10 min and 30 min after stimulation, respectively (Figure 5). A good positive correlation between the amounts of catecholamine and adenine nucleotides appearing in the effluents of each collection period was obtained ($r = 0.92$, $P < 0.01$). There was no difference between the molar ratios of catecholamine to adenine nucleotides with different types of stimulation (splanchnic nerve stimulation, 8.27 ± 0.87, $n = 8$; ACh, 10.43 ± 1.54, $n = 5$; excess K, 9.72 ± 0.71, $n = 5$; veratridine, 12.42 ± 1.6, $n = 16$; mean ± s.e.). The mean molar ratio was 10.75 ± 0.85 (mean ± s.e., $n = 34$). The resting adenine nucleotide values for an initial 10 min collection period tended to be high, even when adenine nucleotides originating from blood were subtracted. Thus the ratio of catecholamine to adenine nucleotides was always lower than that during stimulation. There was no correlation between the amounts of adenine nucleotides and catecholamine found in the resting period.

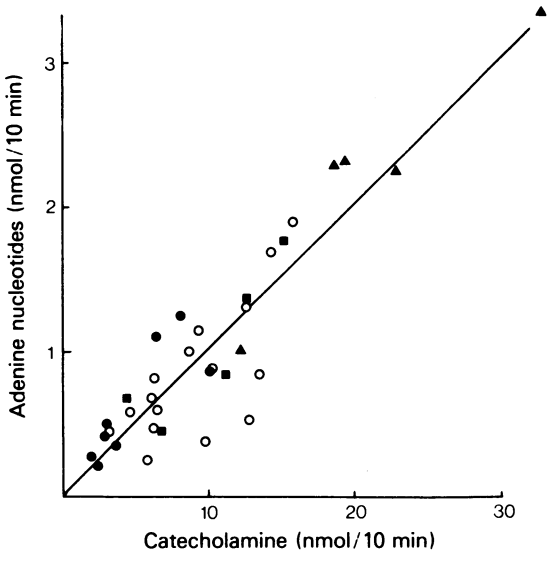

Figure 5 Correlation between the evoked release of catecholamine (nmol) and adenine nucleotides (nmol) for each 10 min collection period. Symbols indicate the amounts of catecholamine and adenine nucleotides induced by splanchnic nerve stimulation (●), infusion of acetylcholine (■), excess K^+(▲) and veratridine (○). The line was computer fitted (correlation coefficient = 0.92, $P < 0.01$).

Discussion

In previous experiments, we found that veratridine caused secretion of catecholamine by activating tetrodotoxin-sensitive Na^+ channels on chromaffin cell membranes in guinea-pig adrenal glands (Ito *et al.*, 1978; 1979). The present results indicate that the release of catecholamine induced by veratridine may occur by exocytosis similar to that induced by splanchnic nerve stimulation, ACh and excess K. The evidence for this is that all of these methods of stimulation cause secretion of catecholamine and DBH activity in the venous effluents in the same proportion

as they are found stored within the chromaffin granules as well as a parallel secretion of adenine nucleotides and catecholamine.

According to Hillarp & Thieme (1959), chromaffin granules contain catecholamine and adenine nucleotides at a constant molar ratio. ATP seems to be degraded during its passage through the adrenal circulation in the perfused cat (Douglas & Poisner, 1966b) and bovine (Banks, 1966) adrenal glands. In pefused cat adrenal gland, catecholamine and AMP caused a strictly parallel rise and fall during splanchnic nerve stimulation and the molar ratio of catecholamine to AMP was 6.1 ± 0.3, but the ratio become 4.22 ± 0.07 if the amounts of AMP plus adenosine were compared with these of catecholamine (Douglas & Poisner, 1966a). Therefore a higher molar ratio of catecholamine to adenine nucleotides in the present experiments (10.75 ± 0.85) may be explained by a further decomposition of adenine nucleotides in their passage through the circulation of guinea-pig adrenal glands.

DBH is contained in chromaffin granules as a granular 'membrane bound' form and a 'soluble' form. In agreement with the report by Arnaiz *et al.* (1978) the 'soluble' DBH activity in the granular fraction of guinea-pig adrenal glands was found to be 62% of the total DBH activity in the present experiments. The 'soluble' DBH is believed to be expelled into the extracellular space with catecholamine. In fact, the ratio of catecholamine to DBH found in the venous effluents following stimulation was similar to that found in chromaffin granules. However, the time course of the secretion of DBH was different from that of catecholamine with all methods of stimulation. It did not result from either the presence of endogenous inhibitors or delay of DBH passing through the adrenal circulation. The release of chromogranin A (Blaschko *et al.*, 1967; Kirshner *et al.*, 1967) and DBH (Dixon *et al.*, 1975; Jacobs *et al.*, 1978; Sorimachi & Yoshida, 1979) in perfusates is also reported to lag behind that of catecholamine. Histologically, secretory granules of adrenal chromaffin cells are released into the space between the plasma membrane and the basal lamina, but the granular profile is no longer visible beyond the basal lamina (Grynszpan-Winograd, 1975). Thus the basal lamina may act as a diffusion barrier for granular contents. This may be the reason why the time course of the secretion of catecholamine and adenine nucleotides was parallel but that of DBH (mol. wt. 290,000 dalton) was slower.

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