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# EXOCYTOTIC RELEASE OF CATECHOLAMINES AND DOPAMINE-β-HYDROXYLASE FROM THE PERFUSED ADRENAL GLAND OF THE RABBIT AND CAT

MASARU SORIMACHI & KENICHI YOSHIDA

Department of Physiology, Ehime University Medical School, Ehime 791-02, Japan

**1** Secretion of catecholamines (CA) and dopamine-β-hydroxylase (DBH) activity from the perfused rabbit and cat adrenal gland was studied following stimulation by a number of substances, including the physiological transmitter, acetylcholine (ACh), added to the perfusion fluid.

**2** Stimulation caused a proportional secretion of DBH and CA from the untreated rabbit adrenal. The ratio of DBH/CA was 11.2 ± 0.9 (mean ± s.e.) which was close to that found in the crude granule fraction of the contralateral gland (11.4 ± 0.7).

**3** After treatment with insulin (40 u/kg) for 3 h or with reserpine (0.25 mg/kg) for 24 h, CA and DBH contents decreased in a parallel fashion in the granule fraction, thus resulting in a similar ratio of DBH/CA in the gland. The ratio in the effluents after stimulation was similar to that found in the untreated gland.

**4** Higher doses of reserpine (0.7 to 2.5 mg/kg) increased the ratio of DBH/CA in the granule fraction and was dependent on the doses employed. The ratio in the effluents from these glands after stimulation paralleled these increased values.

**5** Concomitant secretion of CA and DBH in response to stimulation was also observed in the perfused cat adrenal. However, the ratio of DBH/CA in the effluents tended to be lower than that found in the granule fraction.

**6** These results support the concept of exocytotic secretion of CA in the adrenal medulla.

## Introduction

It has been well established that the release of catecholamines (CA) from the adrenal medulla occurs by exocytosis, that is, the entire soluble content of the chromaffin granules is discharged directly into the extracellular space. Thus, a proportional release of CA and adenosine triphosphate (ATP) was demonstrated in the perfused cat adrenal gland (Douglas, Poisner & Rubin, 1965; Douglas & Poisner, 1966). Secretion of CA from the bovine adrenal gland in response to secretagogues was also accompanied by release of chromogranin and ATP in such a manner that the ratios of CA to these substances were quite similar to their respective ratios in the soluble lysate of chromaffin granules (Blaschko, Comline, Schneider, Silver & Smith, 1967; Schneider, Smith & Winkler, 1967; Kirshner, Sage & Smith, 1967; Lastowecka & Trifaro, 1974).

On the other hand, further studies are required to elucidate the quantitative secretion of dopamine-β-hydroxylase (DBH). Although an earlier report demonstrated a similar ratio of DBH/CA in the effluents to that in the gland, care has to be taken that total DBH activity (soluble and membrane-bound form of the enzyme) is included in the calculation (Viveros, Arqueros & Kirshner, 1968). A recent paper demonstrated that splanchnic nerve stimulation or acetylcholine (ACh) administration evoked a barely detectable release of DBH from the perfused cat adrenal despite releasing massive amounts of CA (Dixon, Garcia & Kirpekar, 1975). To acquire further information on the secretion of DBH, we carried out comparative studies employing perfused cat and rabbit adrenal glands with special attention focused on the quantitative aspects.

## Methods

*Perfusion of cat and rabbit adrenal gland* in situ

Rabbits (2 to 3 kg) and cats (1.5 to 6.0 kg) were anaesthetized with urethane (1 g/kg, i.v.) and Nembutal (40

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mg/kg, i.p.), respectively. The left adrenal gland was perfused at room temperature (25 to 30°C) through a polyethylene cannula inserted into the abdominal aorta with a modified Locke solution of the following composition (mM): NaCl 150, KCl 5, $CaCl_2$ 2, Na phosphate buffer (pH 7.2) 3 and glucose 11. This solution was equilibrated with $O_2$. The venous effluent was collected through a polyethylene cannula inserted into the inferior vena cava (rabbits) or adrenal vein via the inferior vena cava (cats). The flow rate was adjusted to 0.5 to 0.6 ml/min (rabbits) and 0.6 to 1.0 ml/min (cats) by changing the perfusion pressure.

To stimulate secretion, the perfusion was changed for periods of 5 min to the same medium containing ACh (0.1 to 0.5 mM), $BaCl_2$ (2 mM), KCl (60 mM, isotonicity was maintained by reduction of NaCl) or pilocarpine (1 mM). Each 5 min effluent before, during and after stimulation was collected in an ice-chilled measuring cylinder, and samples were divided into 2 parts: one for DBH assay was transferred to test tubes containing bovine serum albumin (BSA, final concentration of 0.25%), and the other for CA assay to test tubes containing 0.01 ml of 5 N acetic acid. In some experiments, in which attempts were made to deplete the glands maximally, 5 min stimulation with $K^+$ 60 mM medium was repeated at 5 min intervals up to 10 times.

*Subcellular fractionation of adrenal glands*

Immediately after the start of the perfusion, the right adrenal gland was excised and kept in chilled 0.3 M sucrose. The gland was chopped and homogenized with a Teflon glass homogenizer in 0.3 M sucrose. In some experiments with cat adrenal glands, the left gland was also excised at the end of the perfusion and homogenized. The homogenate was centrifuged at 1,000 *g* for 10 min and the supernatant decanted. The pellet was resuspended in 0.3 M sucrose and centrifuged again at 1,000 *g* for 10 min. With rabbit gland, the combined supernatant was centrifuged at 26,000 *g* for 20 min. The pellet was dispersed in chilled 5 mM Tris-HCl buffer (pH 7.2), left for more than 30 min at 4°C and centrifuged at 200,000 *g* for 30 to 60 min (Hitachi ultracentrifuge). The resultant supernatant contained CA and soluble DBH derived from the chromaffin granules. In cat gland, the combined supernatant was divided into 2 parts and centrifuged at 26,000 *g* for 20 min. The pellet from one portion was hypo-osmotically treated as for the rabbit adrenal. The other pellet was suspended in 1.5 to 2.0 ml of 0.3 M sucrose, and 1.0 to 1.5 ml was layered over discontinuous sucrose density gradient consisting of 1 ml each of 1.4 M, 1.5 M and 1.6 M sucrose, and then centrifuged at 165,000 *g* for 2 h. The pellet, which contains a highly pure form of the chromaffin granules, was osmotically shocked with 5 mM Tris-HCl buffer and centrifuged at 200,000 *g* for 30 min. The resultant supernatant contained CA and soluble DBH and the pellet contained DBH bound to the granule membrane. In some cases, the 26,000 *g* supernatant was centrifuged at 200,000 *g* for 60 min to obtain a microsomal pellet. The pellet was homogenized with 5 mM Tris-HCl buffer, left for more than 30 min and centrifuged at 200,000 *g* for 90 min. The resultant supernatant was saved for the assay of CA and DBH. The pellet was sonicated in the same buffer for 30 to 40 s (Kontes) and centrifuged at 200,000 *g* for 90 min, the resultant supernatant being saved. When necessary, this sonication procedure was repeated.

Any material containing CA and the soluble DBH was divided into 2 parts; one part was acidified with 1/10 volume of 0.2 N perchloric acid for CA assay, and the other was dialysed in the presence of BSA (final 0.25%) and freeze dried. There was negligible inactivation of CA during relatively long periods of ultracentrifugation, since CA content in the suspension of 26,000 *g* pellet was unchanged when analyzed before and after centrifugation at 200,000 *g* for 60 min.

*Analytical procedure*

CA was measured fluorimetrically by the conventional trihydroxyindole method (Anton & Sayre, 1962). Effluents and tissue samples were in most cases assayed without purification on alumina, although inclusion of this step did not alter the results. Differential determination of adrenaline and noradrenaline was based on the differential oxidation of the two amines at pH 7 and pH 3.5. When the amounts of CA were too low to be measured accurately with a fluorimetric procedure (e.g. after treatment with higher doses of reserpine), a radiochemical assay was performed according to the method of Coyle & Henry (1973). The only modification was that catechol-*O*-methylated products were extracted with a mixture of toluene and isoamylalcohol (3:2). Internal standards of CA were always included.

DBH activity was measured radiochemically by the method of Molinoff, Weinshilboum & Axelrod (1971) which involves a two-step coupled reaction. Phenylethylamine is converted to phenylethanolamine by DBH at pH 5.5 in the first step, followed by *N*-methylation of this product in the presence of partially purified phenylethanolamine-*N*-methyltransferase (PNMT) and radioactive S-adenosyl-L-methionine. Before assay, effluents and tissue samples were routinely dialyzed for 24 h against 5 l of 2 mM Tris-HCl buffer (pH 7.2) with change of medium once, and then freeze dried. After dialysis, the concentration of Na ions in the effluents was reduced to less than 1/500 when measured by atomic absorption analysis.

Inclusion of an internal standard (phenylethanolamine) indicated that in dialyzed samples there was no PNMT inhibitor. In order to ascertain whether any loss in enzyme activity occurred during dialysis and freeze drying, previously dialysed soluble DBH containing comparable activity to that found in the effluents was treated in the same way as the samples. The recovery was 80 to 95% and all sample DBH activity was routinely corrected for recovery. Boiled effluents, which were dialysed in the presence of BSA and then freeze dried, served as a blank. Boiling the sample in the presence of BSA gave a somewhat higher blank value.

Various concentrations of copper ions were, when necessary, included in the first DBH reaction to inactivate DBH inhibitors. Although copper was usually unnecessary for dialyzed effluents and tissue samples containing soluble DBH, additional assays including copper at 1.7 to 3.3 μM were routinely performed to ensure maximal activity. On the other hand, inclusion of copper was necessary to obtain the maximal activity of membrane bound DBH, which was solubilized by sonication in presence of Triton X-100 or Cutscum (Fisher Scientific Co.).

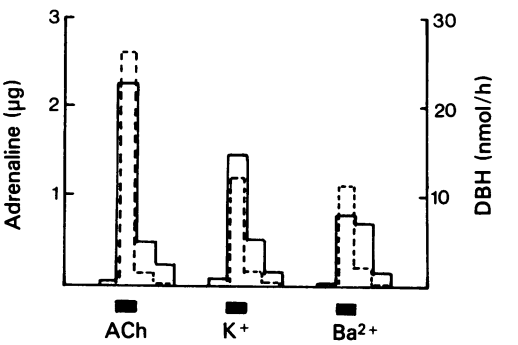


**Figure 1** Release of dopamine-β-hydroxylase (DBH) and adrenaline from the perfused rabbit adrenal by acetylcholine (ACh, 0.1 mM), $K^+$ (60 mM) and $Ba^{2+}$ (2 mM). The heights of the columns represent the amounts of DBH (continuous line) and adrenaline (dashed line) in effluents collected for each 5 min. The filled squares represent the period during which the gland was perfused with medium containing stimulating agents.

## Results

*The ratio of soluble dopamine-β-hydroxylase to adrenaline in the 26,000 g pellet*

The exocytotic theory of secretion requires that the ratio of soluble DBH to CA in the effluents should be similar to that found in the adrenal gland. Therefore the content of soluble DBH in the chromaffin granules had to be measured precisely. Soluble DBH in the 26,000 *g* pellet of a non-perfused contralateral gland could be considered equivalent to that in the chromaffin granules and most of it may be released after osmotic lysis of the granule since more than 95% of adrenaline was recovered in the supernatant after ultracentrifugation. In the rabbit gland, approximately 15 to 20% (mean 16.9%) of DBH activity found in this fraction was soluble and values varied from 191 to 670 nmol phenylethanolamine formed/h per gland with a mean of 429.6 ($n = 10$). Over this wide range, adrenaline content paralleled the activity of soluble DBH and the mean ratio of soluble DBH (nmol/h) to adrenaline (μg) was 11.4 ± 0.7 (mean ± s.e., $n = 10$). The ratios in cat adrenal gland were 1.90 ± 0.12 ($n = 11$) and 1.16 ± 0.26 ($n = 6$) in the 26,000 *g* pellet and in the purified granules obtained after density gradient, respectively. In the latter, the soluble DBH was approx. 20% of the total DBH.

*Release of adrenaline and dopamine-β-hydroxylase from the untreated rabbit adrenal gland*

After the start of the perfusion, the secretion of adrenaline and DBH progressively declined and resting values became negligible after 30 to 40 min. Most adrenaline appeared during the 5 min-stimulation period when secretion was evoked by ACh, $K^+$ 60 mM and $Ba^{2+}$, and only a small amount was found in the effluent collected during the 5 min post-stimulation period (Figure 1). On the other hand, the release of DBH lasted for a rather long period, significant amounts being still present in the second 5 min post-stimulation period. The net DBH activity released was therefore calculated to be that released during 5 min stimulation and 10 min post-stimulation periods. When adrenaline and DBH were measurable in the effluent before stimulation, these values were subtracted from each 5 min sample during and after stimulation. The net amounts of DBH released were variable, depending on the glands and the stimulating agents. Over a wide range, the secretion of DBH paralleled that of adrenaline and the mean ratio of DBH/adrenaline was 11.2 ± 0.9 (mean ± s.e., $n = 12$). This value is comparable with that found in the 26,000 *g* pellet (11.4 ± 0.7). Individual ratios in the effluents are plotted against those in the 26,000 *g* pellet obtained from the contralateral gland in Figure 2.

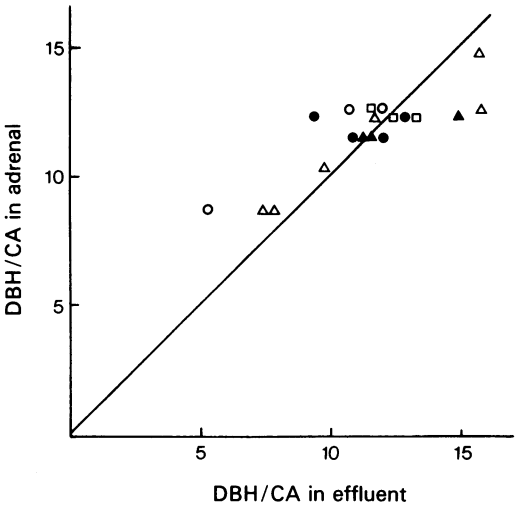


**Figure 2** Correlation between the ratios of dopamine-β-hydroxylase (DBH) to adrenaline in the 26,000 *g* pellet (ordinate scale) and those in the effluents after stimulation (abscissa scale) obtained from rabbit adrenal. The continuous line represents the ideal line obtained if both ratios are correlated 1:1. Open and filled symbols indicate untreated and insulin-treated adrenals, respectively. Adrenal glands were stimulated with ACh (0.1 to 0.5 mM; ○ and ●), $K^+$ (60 mM, △ and ▲) or $Ba^{2+}$ (2 mM, □).

*Release of adrenaline and dopamine-β-hydroxylase from adrenals of rabbits treated with insulin and reserpine*

Insulin (40 units/kg) was given intravenously to 4 rabbits and 3 h later adrenal glands were excised. Adrenaline content and soluble DBH activity in the 26,000 *g* pellet were reduced to 28% (mean) and 33%, respectively, of untreated animals. Two of these animals were used for perfusion experiments. The ratios of soluble DBH/adrenaline in the 26,000 *g* pellet were 11.4 and 12.3. These values are comparable with those obtained in untreated animals, thus confirming that insulin depletes soluble DBH and adrenaline by neurogenic stimulation. This finding also suggests only a minor contribution of immature granules, which have not acquired any adrenaline or are undergoing synthesis, to the total population of chromaffin granules, since one would expect that otherwise there would be an increased ratio of soluble DBH to adrenaline after intense secretory activity. Consistent with this observation, the ratios in the effluents after stimulation with ACh and $K^+$ 60 mM were comparable with those in the untreated animals. These ratios are included in Figure 2.

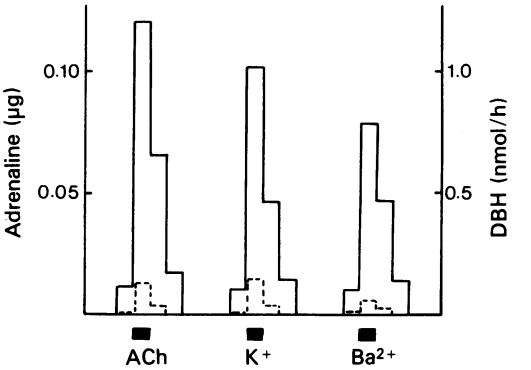


**Figure 3** Release of dopamine-β-hydroxylase (DBH) and adrenaline from the perfused rabbit adrenal treated with reserpine (2.5 mg/kg) for 24 h. Adrenaline shown by dashed line and DBH by continuous line. For further explanation see legend to Figure 1.

Similar results were obtained in two glands treated for 24 h with lower doses of reserpine (0.25 mg/kg, i.p.). The ratio in the effluents after stimulation ranged from 10.2 to 14.0 (mean 11.6), values close to those in the 26,000 *g* pellet (10.7; 12.9), suggesting that this dose of reserpine, like insulin, neuronally stimulated the glands. On the other hand, administration of larger doses (0.7 to 2.5 mg/kg) to animals increased the ratios of soluble DBH to adrenaline in the 26,000 *g* pellet, that is, the depletion of adrenaline was much greater than that of soluble DBH. Concomitant with increased ratios of 17.1 and 17.3 observed in two glands from animals treated with reserpine (0.7 mg/kg), the ratio increased to 15.3 to 18.1 in the effluents after stimulation. Further increases in the dose resulted in higher ratios in both the 26,000 *g* pellet and the effluents after stimulation. The highest dose (2.5 mg/kg) severely depleted adrenaline to 0.12 μg/gland and soluble DBH to 18 nmol/h per gland, resulting in the ratio of 150. An increased secretion of adrenaline and DBH was still observed after perfusion of stimulating agents and the ratio DBH/adrenaline ranged from 56 to 123 (Figure 3). These results are included in Figure 4.

*Release of catecholamines and dopamine-β-hydroxylase from the cat adrenal*

The secretion pattern of CA and DBH was quite similar to that found in the rabbit adrenal (Figure 5). The correlation between the ratio of DBH to CA in the effluents and that in the adrenals (26,000 *g* pellet) was not as satisfactory as that found in the rabbit experiments (Figure 6).

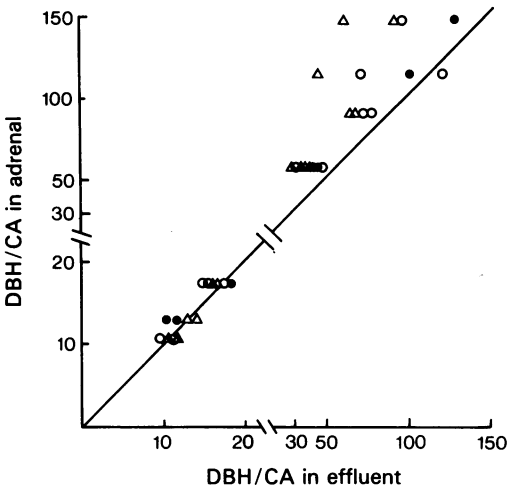


**Figure 4** Correlation between the ratios of dopamine-β-hydroxylase (DBH) to adrenaline in the 26,000 *g* adrenal pellet (ordinates) and the effluents (abscissae) obtained after stimulation from rabbits treated with various doses of reserpine. The continuous line represents the ideal line obtained if these ratios were correlated 1:1. (○) and (●) represent values obtained after stimulation with acetylcholine (0.1 to 0.5 mM) and $Ba^{2+}$ (2 mM), respectively; (△) represent values obtained by $K^+$ 60 mM stimulation. Note that the ordinate and abscissa scales are changed at the break in the lines.

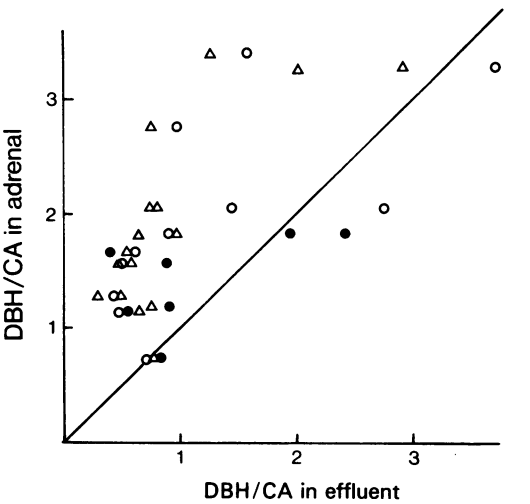


**Figure 6** Correlation between the ratios of dopamine-β-hydroxylase (DBH) to total CA (adrenaline plus noradrenaline) in the 26,000 *g* adrenal pellet (ordinates) and the effluents (abscissae) obtained from the cat adrenal after stimulation. The continuous line represents the ideal line obtained if these ratios were correlated 1:1. Adrenal glands were stimulated with acetylcholine (0.1 mM, ○), pilocarpine (1 mM, ●) or $K^+$ (60 mM, △).

Irrespective of the predominant amine secreted by different stimulating agents, a similar ratio of soluble DBH to total CA was observed in the effluents after stimulation (Table 1), indicating the similar activity of soluble DBH per unit of individual amine in the adrenaline and noradrenaline granules. Thus, the higher ratio of soluble DBH to adrenaline (11.4 ± 0.7) in adrenaline granules in rabbit adrenal is species-dependent and thus this cannot be responsible for the poor correlation observed in the cat adrenal.

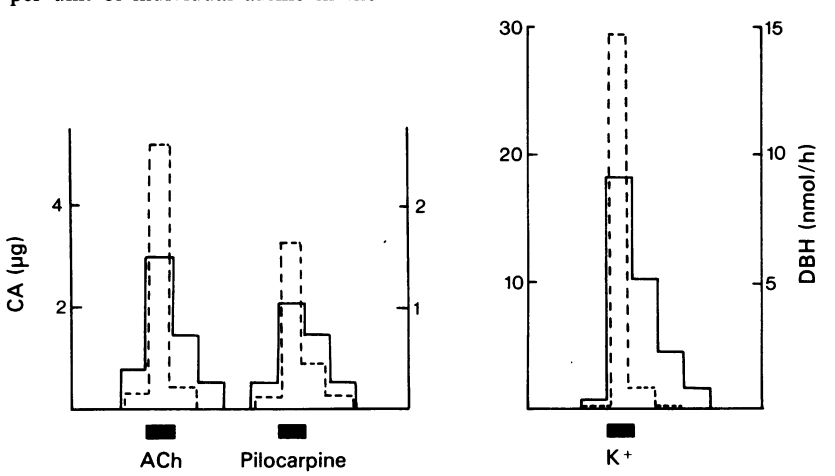


**Figure 5** Release of dopamine-β-hydroxylase (DBH, continuous line) and adrenaline (dashed line) from the perfused cat adrenal by acetylcholine (ACh, 0.1 mM), pilocarpine (1 mM) and $K^+$ (60 mM). For further explanation see legend to Figure 1.

**Table 1** The differential secretion from the cat adrenal gland of noradrenaline and adrenaline induced by pilocarpine, acetylcholine (ACh) and KCl and its relation to the ratio of dopamine-β-hydroxylase (DBH) to total catecholamines (CA)

| | *Effluents* | | |
|---|---|---|---|
| *Stimulating agents* | *Noradrenaline (%) of total CA* | *DBH/CA* | *26,000 g pellet DBH/CA* |
| Pilocarpine (1 mM) | 19.4 ± 4.8 (6) | 0.65 ± 0.10 (6) | 1.26 ± 0.13 (6) |
| ACh (0.1 mM) | 42.7 ± 3.2 (7) | 0.60 ± 0.06 (7) | 1.34 ± 0.13 (7) |
| KCl (60 mM) | 68.0 ± 5.8 (13) | 0.65 ± 0.05 (13) | 1.42 ± 0.13 (9) |

For simple comparison, these values are obtained from the adrenals, in which the ratios of DBH to CA in 26,000 *g* pellet are less than 1.8. Numbers of experiments are indicated in parentheses.

**Table 2** Comparison of the ratios of soluble dopamine-β-hydroxylase (DBH) to total catecholamines (CA) in the 26,000 *g* pellet and the density gradient pellet from cat adrenal glands

| | *DBH/CA* | |
|---|---|---|
| *Experiment no.* | *26,000 g pellet* | *Density gradient pellet* |
| 1 | 1.27 | 1.30 |
| 2 | 1.55 | 1.27 |
| 3 | 1.94 | 1.64 |
| 4 | 1.55 | 1.13 |
| 5 | 0.72 | 0.78 |
| 6 | 1.15 | 0.81 |
| Mean | 1.36 | 1.16 |

To determine whether or not inactivation of DBH occurred within the gland after secretion, a dialyzed preparation of soluble DBH obtained from a 26,000 *g* pellet was perfused through the gland, and the DBH activity was measured in the effluents. Recovery of DBH was 85 and 90% in two experiments.

*The ratio of soluble dopamine-β-hydroxylase to catecholamines in the density gradient pellet from cat adrenal glands and the effect of repeated stimulation*

The ratios of soluble DBH and CA were compared between the 26,000 *g* pellet and the density gradient pellet in the individual gland in order to determine whether or not the value in the 26,000 *g* pellet differs greatly from that in the pure granules (Table 2). The difference was quite small, though a tendency towards a lower ratio was observed in the pure granules. It is possible that the density gradient pellet still contained different populations of granules and only one population was concerned with the secretion. This possibility was also examined and we attempted to deplete all CA by repeated stimulation with $K^+$ 60 mM, and to compare the ratios of soluble DBH and CA between the stimulated and the non-stimulated glands (Table 3). Total CA was reduced to the mean of 44% of the contralateral gland in 4 stimulated glands. Since $K^+$ 60 mM rather selectively causes the secretion of noradrenaline (Rubin & Miele, 1968), the depletion of noradrenaline was greater (mean 75%) than that of adrenaline (mean 43%), thus resulting in an increase in the ratio of adrenaline/noradrena-

**Table 3** The effect of repeated stimulation by $K^+$ 60 mM on the ratios of soluble dopamine-β-hydroxylase (DBH) to catecholamines (CA) in the 26,000 *g* pellet and in the density gradient pellet from cat adrenal glands

| | *Non-perfused control adrenal* | | *Stimulated adrenal* | |
|---|---|---|---|---|
| | *DBH/(Ad + NA)* | *Ad/NA* | *DBH/(Ad + NA)* | *Ad/NA* |
| 26,000 *g* pellet | 1.19 ± 0.18 (4) | 1.18 ± 0.16 (4) | 1.29 ± 0.18 (4) | 3.28 ± 0.63* (4) |
| Density gradient pellet | 0.91 ± 0.11 (3) | 0.63 ± 0.12 (3) | 0.89 ± 0.02 (3) | 1.68 ± 0.06** (3) |

Ad = adrenaline; NA = noradrenaline. Numbers of experiments indicated in parentheses.
* $P < 0.02$; ** $P < 0.01$.

line. The increase in the ratio of adrenaline/noradrenaline was less noticeable in the density gradient pellet, possibly because of the lower recovery of the adrenaline granules in the pellet, since their density is lower than that of the noradrenaline granules (Schümann, 1957; Eade, 1958). Irrespective of the DBH source, the ratio was not significantly different from that in non-perfused control gland (Table 3). Since the DBH activity per individual amine is comparable in the two kinds of granules (Table 1), both pellets could be considered to comprise practically one population of granules in terms of the ratio of soluble DBH/CA.

*The soluble dopamine-β-hydroxylase activity in microsomal fraction of control and stimulated cat adrenal glands*

Morphological studies have demonstrated the presence of coated pits in the granule membrane after its fusion-fission with the plasma membrane (Grynszpan-Winograd, 1971; Douglas, Nagasawa & Schulz, 1971) and the uptake of exogenously administered thorium dioxide into the coated vesicles (Nagasawa & Douglas, 1972). This suggested that following exocytosis, the granule membrane is retrieved into the cells by the process of vesiculation. This, together with the fact that DBH secretion lags behind that of CA (Figure 4), raised the question of whether or not some of the DBH secreted from one cell is reincorporated into the cell thus resulting in a decrease of DBH in the effluents. If so, most of these DBH molecules would be found in the microsomal fraction of the stimulated glands. However, no increase in DBH activity was found in lysates of microsomal pellets of the stimulated gland when compared with those derived from non-stimulated glands (Table 4). On the other hand, an increase in the activity was evident in the pellets, a fact which indicates an increased number of membrane fragments of emptied granules following exocytosis. To ensure that any trapped enzymes in the small vesicles would not be released upon simple osmotic shock, the resultant pellets were sonicated and the released DBH activity was measured. The DBH activity had increased in the stimulated gland to twice that of the unstimulated gland, but the additional sonication of the resultant pellets further released DBH (Table 4). This indicates that membrane-bound enzymes can be partly solubilized by sonication even in the absence of detergents.

## Discussion

Viveros *et al.* suggested the exocytotic secretion of CA and DBH in rabbit adrenal medulla by showing that neurogenic stimulation by insulin hypoglycaemia caused a decrease in both the soluble DBH activity and CA content of the chromaffin granule fraction (Viveros, Arqueros, Connett & Kirshner, 1969a; Viveros, Arqueros & Kirshner, 1969b). Our perfusion study provides further evidence for this mechanism of secretion. Stimulation of the untreated rabbit glands by perfusion of stimulating agents resulted in a concomitant secretion of DBH and adrenaline into the venous effluents in such a way that the ratio DBH/adrenaline was close to that found in the granule fraction (Figure 2). The same was found in the adrenals treated with insulin or reserpine. Treatment with insulin or the lowest dose of reserpine employed, depleted both the soluble DBH and adrenaline from the granule fraction, but the ratio remained similar to that found in the untreated gland. In these glands, DBH and adrenaline appeared in the effluents after stimulation and the ratio was similar to that found in the untreated gland. On the other hand, administration of higher doses of reserpine increased the ratio of soluble DBH to adrenaline in

**Table 4** Solubilization of dopamine-β-hydroxylase (DBH) from the microsomal pellet of control and stimulated cat adrenal glands by hypo-osmotic shock or sonication

| *Experiment no.* | *Treatment* | *Soluble DBH* | | *Membrane DBH* | |
|---|---|---|---|---|---|
| | | *Control* | *Stimulated* | *Control* | *Stimulated* |
| 1 | Osmotic shock | 11.2 | 9.3 | 320 | 577 |
| 2 | Sonication | 27.8 | 67.3 | 174 | 357 |
| 3 | Osmotic shock | 7.8 | 6.8 | | |
| | Sonication (1) | 29.8 | 63.0 | | |
| | (2) | 11.6 | 18.7 | | |
| | (3) | 16.4 | 31.8 | 230 | 721 |

DBH activity is expressed as nmol product formed/h per gland. In experiment 3, the microsomal pellet was hypo-osmitically treated and the pellet after centrifugation was sonicated in 5 mM Tris buffer pH 7.2. The pellet after centrifugation was again sonicated and this procedure was repeated once.

the granule fraction. The additional depletion of adrenaline could be due to the inhibitory effect of reserpine on the transport of adrenaline through the granule membrane (Viveros *et al.*, 1969a). A good correlation was also found in these glands between the ratios of soluble DBH/adrenaline in 26,000 *g* pellet and those in the effluents (Figure 4) suggesting that the secretion of granule content is quantal in nature. It is of interest that there was still a significant increase in adrenaline and DBH secretion after stimulation from the adrenal in which more than 99% of adrenaline and approx. 95% of soluble DBH were depleted after the highest dose of reserpine (Figure 3). This confirms the observations by Dixon *et al.* (1975) that chromaffin granules depleted of CA continue to participate in the secretory cycle and further suggests that the gland secretes its content of granules until the store is completely exhausted.

Dixon *et al.* (1975) did not demonstrate a proportional secretion of DBH and CA from the cat adrenal, though their fractionation studies supported the concept of exocytosis. We therefore perfused the cat adrenal as well and found, in contrast to their results, a concomitant secretion of both in response to stimulating agents (Figure 5). Secretion patterns of both were quite comparable with those found in the perfused rabbit adrenal. The discrepancy between their results and ours can probably be ascribed to differences in techniques employed (e.g. perfusion method, dialysis of the effluents). However, in our preparations, the amounts of DBH secreted were still less than that expected from the ratios found in the glands, though in some experiments a corresponding secretion of DBH and CA was found (Figure 6). No definite explanation can be given at present. However, two further possibilities may be considered, and are probably species-dependent. One is that there is a non-dialyzable inhibitor(s) of DBH in the effluents, that originates in the cortical tissue and could not be eliminated by the addition of copper ions. The other is that part of the DBH secreted is reincorporated into the cell, followed by the inactivation of this enzyme by the lysosomal enzymes, since Nagasawa & Douglas (1972) suggested that in the hamster adrenal medulla, coated or smooth vesicles containing exogenous thorium dioxide are readily taken up into the lysosomes.

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