Differential secretion of catecholamines in response to peptidergic and cholinergic transmitters in rat adrenals

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1. Rat adrenal medulla is stimulated by cholinergic and peptidergic transmitters released from splanchnic nerves. The peptidergic transmitter has been identified as vasoactive intestinal polypeptide (VIP) and its contribution in comparison to that of acetylcholine (ACh) is more prominent at low neuronal activity. The purpose of this study is to determine if ACh and VIP cause differential secretion of adrenaline and noradrenaline and whether the differential secretion also occurs when splanchnic nerves are stimulated at different frequencies.

2. Perfusion of the left adrenal gland with Krebs solution for several hours did not change adrenaline and noradrenaline contents (15·2 μg and 3·5 μg, respectively) and their ratio (4·4) from those of the unperfused right adrenal medulla (15·2 μg, 3·3 μg and 4·8, respectively).

3. Perfusion with ACh (10 μM for 4 min) resulted in the secretion of 109 ng of catecholamines and the ratio of adrenaline to noradrenaline was 3·8. Although the secretion increased with increased concentrations of ACh (30 and 100 μM), the ratios remained between 3 and 4.

4. Perfusion with VIP (10 μM for 4 min) resulted in the secretion of 27 ng of catecholamines and the ratio of adrenaline to noradrenaline was 9·7. A higher concentration of VIP (20 μM for 4 min) resulted in the secretion of greater amounts of catecholamines (102 ng) without significantly altering the ratio of adrenaline to noradrenaline (10·9).

5. Perfusion with as low as 0·01 μM pituitary adenylate cyclase-activating polypeptide (PACAP) increased the secretion of catecholamines to 31 ng and the secretion increased in a dose-dependent manner up to 0·3 μM. The ratio of adrenaline to noradrenaline was 6·8 with 0·1 μM PACAP and remained almost unchanged throughout the concentration range.

6. Electrical field stimulation at 0·5 Hz for 300 s resulted in the secretion of 34 ng catecholamines, and the ratio of adrenaline to noradrenaline was 6·9. Delivering the same number of pulses at 1 Hz increased the secretion to 42 ng but reduced the ratio to 6·3. Increasing stimulation frequency to 10 Hz for 15 s or 30 s further increased the secretion to 66 ng and 180 ng, respectively, but significantly reduced the ratio to about 5.

7. Delivery of same number pulses at 3 Hz continuously or at 30 Hz in bursts caused a substantial reduction in catecholamine secretion (55 *versus* 35 ng) and in the ratio of adrenaline to noradrenaline (7·4 *versus* 5).

8. These results agree with the hypothesis that cholinergic and peptidergic transmitters control catecholamine secretion at different levels of neuronal activity and provide new evidence that ACh stimulates the secretion of noradrenaline and adrenaline whereas VIP and PACAP predominantly stimulate the secretion of adrenaline.

Adrenal chromaffin cells synthesize and store large amounts of catecholamines. In most mammalian adrenal glands the catecholamines are predominantly adrenaline and noradrenaline with dopamine making up only a very small proportion of the total. There is sufficient evidence that adrenaline and noradrenaline are synthesized and stored in separate chromaffin cells (Hillarp &Hökfelt, 1953; Eränkö, 1955; Coupland, Pyper & Hopwood, 1964; Goldstein, Fuxe, Hökfelt & Joh, 1971; Schultzberg, Andersson, Undén, Troye-Bolmberg, Svenson & Bartfai, 1989). The proportions of adrenaline and noradrenaline vary from species to species. Even in the case of the rat adrenal gland the ratio of

adrenaline to noradrenaline varies from 2·5 to 5 (Eränkö & Raisanen, 1957; Tischler *et al.* 1982; Wakade & Wakade, 1983; Tomlinson, Durbin & Coupland, 1987). The physiological significance for the presence of two types of catecholamine has not been examined in detail, although there is some evidence that different proportions of adrenaline and noradrenaline are secreted *in vivo* during stress induced by chemical agents (Feuerstein & Gutman, 1971; Khalil, Livett & Marley, 1986). There is also some evidence from the isolated adrenal gland of the cat that stimulation of acetylcholine muscarinic receptors results mostly in the secretion of adrenaline whereas stimulation of acetylcholine nicotinic receptors results in secretion of noradrenaline and adrenaline (Douglas & Poisner, 1965; Rubin & Miele, 1968).

Acetylcholine is the well-recognized cholinergic transmitter involved in stimulation of the adrenal medulla (Dale, 1914; Feldberg, Munz & Tsudzimura, 1934). Recently, a peptidergic neurotransmitter identified as vasointestinal polypeptide (VIP) has also been shown to stimulate the adrenal chromaffin cells. Acetylcholine (ACh) and VIP differentially participate in stimulation of chromaffin cells at different levels of splanchnic nerve activity. The contribution of VIP is more prominent at low rates of neuronal activity whereas ACh is a major contributor at higher activity (Malhotra & Wakade, 1987; Wakade, Blank, Malhotra, Pourcho & Wakade, 1991). The concept of dual neurotransmitter control of catecholamine secretion raises one important question: why two separate neurotransmitters are required to achieve the same physiological response, namely the secretion of catecholamines. An interesting possibility, which has not yet been tested, is that cholinergic and peptidergic transmitters could differentially stimulate adrenaline-containing cells compared to noradrenaline-containing cells. Therefore one of our goals was to determine the ratios of adrenaline and noradrenaline secreted in the perfusate of the isolated rat adrenal gland stimulated by exogenous ACh and VIP. Another goal was to compare the ratios of adrenaline to noradrenaline after electrical stimulation of splanchnic nerves at different frequencies, the rationale being that if cholinergic and peptidergic transmitters are released in varying proportions then the ratios of adrenaline to noradrenaline should differ at low and high frequency stimulation.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a new member of the secretin–glucagon–VIP family and is biochemically related to VIP (Miyata *et al.* 1989). PACAP immunoreactivity and PACAP-specific binding site have been detected in the rat adrenal gland (Arimura, Somogyävri-Vigh, Miyata, Mizuno, Coy & Kitada, 1991; Shivers, Görcs, Gottschall & Arimura, 1991; Watanabe *et al.* 1992). Therefore, it was of special interest to see if PACAP could stimulate secretion of catecholamines and if the ratios of adrenaline to noradrenaline would be comparable to those produced by VIP. Some of the results were presented to the 22nd annual meeting of the Society for Neuroscience (Guo, Haycock & Wakade, 1992).

METHODS

Perfusion of the adrenal gland

The left adrenal gland of the rat was perfused as described previously by Wakade (1981*a*). Briefly, male Sprague–Dawley rats (250–350 g) were anaesthetized with ether and the abdomen was opened. The left renal vein was cannulated and the tip of the cannula remained near the junction of renal and adrenal veins. All other blood vessels were ligated. Just prior to removal of the adrenal gland, the animal was killed with a dose of ether. The adrenal gland, along with the tied blood vessels and the cannula, was removed from the rat and placed on a metal plate mounted in a Lucite chamber. The metal plate was made up of Ag–AgCl and served as one of the electrodes for stimulation of the gland; another plate electrode was placed on top of the gland. The chamber was maintained at 37 °C by circulating heated water. The gland was perfused at 0·33 ml min^{-1} by means of a motor pump (Sigma, St Louis, MO, USA). The perfusion medium was Krebs–bicarbonate solution of the following composition (mM): Na^+, 143·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·18; Cl^-, 125·6; SO_4^{2-}, 1·2; HCO_3^-, 25; glucose, 11·7. The solution contained Na_2EDTA (10 μg ml^{-1}) to prevent oxidation of catecholamines. The perfusion medium was constantly bubbled with 95% O_2–5% CO_2 and the pH was 7·4 ± 0·1 ($n = 32$). The perfusate escaped from a slit made in the adrenal cortex and was collected in chilled tubes for the analysis of catecholamines.

Stimulation of the adrenal gland

Electrical stimulation of the adrenal gland was achieved by connecting the plate electrodes to a Grass stimulator, Model S88 (Grass Instruments, Quincy, MA, USA). Stimulation parameters were 1·0 ms duration and 100 mA strength. Electrical stimulation was given at 0·5 Hz for 300 s, 1 Hz for 150 s, 3 Hz for 50 s, 10 Hz for either 15 or 30 s, and 30 Hz for 5 s. Burst stimulation was given by delivering 150 pulses at 30 Hz for 1 s at 10 s intervals. Field stimulation selectively activates splanchnic nerves but not the chromaffin cells. Agonist-evoked secretion was achieved by perfusing the gland for 4 min with Krebs solution containing the secretagogue. The interval between each stimulation varied from 10 to 60 min. The same preparation was exposed to different types of stimuli. Secretory responses to electrical stimulations and agonists remain reproducible in the same gland for several hours (Wakade, 1981*a*, *b*).

Collection of perfusate

Prior to each stimulation, either electrical or chemical, perfusates were collected to determine the spontaneous secretion of catecholamines. Immediately after the collection of the 'background sample', collection of the perfusate was continued in another tube, and the adrenal gland was stimulated electrically or chemically. The perfusates were collected for 8 min when 0·5 Hz stimulation was used. In all other experiments the collection time was 4 min. The amounts secreted in the 'background sample' in the same time period were subtracted from those secreted from the 'stimulated sample' to obtain net secretion of catecholamines shown in the figures.

Measurement of catecholamines

The perfusate was analysed for adrenaline and noradrenaline content after high-performance liquid chromatography (HPLC) separation by electrochemical detection (Kissinger, 1977;

Table 1. Ratios of adrenaline to noradrenaline in perfused and unperfused adrenal glands

		Catecholamine content (μg per medulla)		
Experimental condition	*n*	Adrenaline	Noradrenaline	Adrenaline : noradrenaline
Perfused left adrenal gland	10	15·2 ± 1·4	3·5 ± 0·2	4·4 ± 0·4
Unperfused right adrenal gland	23	15·2 ± 0·8	3·3 ± 0·1	4·8 ± 0·3

The left adrenal gland was perfused with Krebs solution and stimulated chemically and electrically for 4–6 h. The unperfused right adrenal gland was placed in ice-cold Krebs solution for the same time. Each value is a mean ± S.E.M.; *n* is the number of experiments.

Wakade & Wakade, 1982). Briefly, perfusate was acidified with 0·8 N perchloric acid to obtain a final normality of 0·1 N. After filtering through a 0·2 μm filter, 20 μl perfusate was injected onto a phase II (100 × 3·2 mm, 3 μm) column and eluted catecholamines were detected using the glossy carbon electrochemical detector. The electrode potential was maintained at 0·7 V *vs.* a Ag–AgCl reference electrode. The mobile phase consisted of 0·1 M monochloracetate buffer at pH 3·1 containing 0·5 mM Na_2EDTA, 0·65 mM octylsulphate and 1·5% (v/v) acetonitrile. The solvent peak was eluted in about 1 min, noradrenaline in about 3 min and adrenaline in about 5 min. Peak heights were measured and calibrated against known concentrations of noradrenaline and adrenaline. After appropriate corrections for dilution, catecholamines in the perfusate were expressed as nanograms noradrenaline and adrenaline per stimulation period. To measure the catecholamines of the adrenal medulla, it was separated from the cortex and homogenized with a glass tissue homogenizer in 0·1 N perchloric acid (1 ml) at 4 °C. The homogenate was diluted 200 times using 0·1 N percholoric acid, filtered and assayed as described above.

Statistics

All the data were presented as means with standard errors and differences were compared using Student's *t* test (two group comparisons) and Fisher's *F* test (multigroup comparisons).

Drugs and their sources

The following drugs were used in these experiments: acetylcholine chloride (Sigma Chemical Co., St Louis, MO, USA); VIP, $PACAP_{38}$ (Bachem California Co., Torrance, CA, USA and Penninsula Laboratories, Belmont, CA, USA). We thank Dr John Haycock for a generous gift of VIP.

RESULTS

Contents of catecholamines in the adrenal medulla

For comparison of ratios of adrenaline to noradrenaline secreted in the perfusate by various stimulation procedures with those in the adrenal medulla, we established the endogenous adrenaline and noradrenaline contents of the adrenal medulla. The average content of adrenaline of ten perfused left adrenal medullae was 15·2 μg and that of noradrenaline was 3·5 μg (Table 1). These contents were very close to those of twenty-three unperfused right adrenal medullae. The ratios of adrenaline to noradrenaline in the perfused left and unperfused right adrenal glands were 4·4 and 4·8, respectively, which are statistically not significant.

Ratios of adrenaline to noradrenaline in perfusates of ACh-stimulated adrenals

Perfusion with 10 μM ACh for 4 min caused a significant increase in the secretion of adrenaline and noradrenaline, and the amounts of both catecholamines increased when ACh concentration increased to 30 and 100 μM (Fig. 1*A*). The ratios of adrenaline to noradrenaline in the perfusate after stimulation with different concentrations of ACh are shown in Fig. 1*B*. The ratio at lower concentrations was 3·8 ± 0·2 ($n = 22$) and did not statistically differ from the ratio with higher concentrations. These ratios were also not statistically different from the ratios of adrenaline to noradrenaline of the adrenal medulla shown in Table 1.

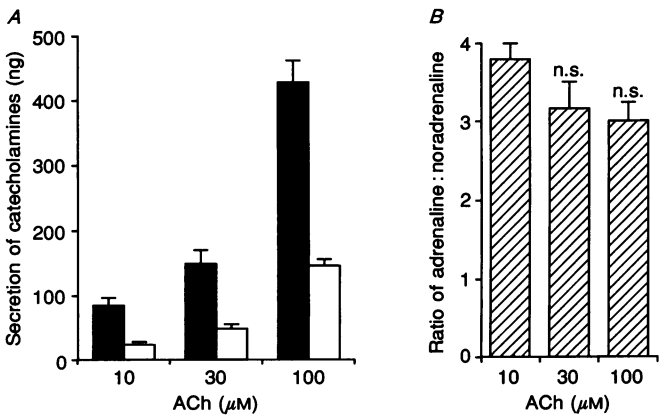

Figure 1. Secretion of catecholamines induced by different concentrations of ACh
Secretion was evoked by introducing each concentration of ACh for 4 min at 10 min intervals in the same adrenal gland. Net secretion of catecholamines (CA) (see Methods) is shown in this and the subsequent figures. Each column represents a mean of 4–22 experiments. Vertical lines show S.E.M. *A*, adrenaline (filled columns); noradrenaline (open columns). *B*, ratios of adrenaline to noradrenaline; n.s., statistically not significant compared with 10 μM ACh.

Table 2. Summary of results showing differential secretion of adrenaline and noradrenaline in response to different agonists and frequencies of stimulation

Agonist or frequencies of stimulation	n	Adrenaline : noradrenaline	Student's t test
ACh (10–100 μM)	36	3·5 ± 0·17	ACh *vs.* VIP $P < 0·001$
VIP (10–20 μM)	20	10·1 ± 0·69	VIP *vs.* PACAP $P < 0·001$
PACAP (0·01–0·3 μM)	40	6·6 ± 2·7	PACAP *vs.* ACh $P < 0·001$
0·5–3 Hz (150 pulses)	27	6·6 ± 0·35	Low frequencies *vs.*
10–30 Hz (150 pulses)	27	5·2 ± 0·26	high frequencies $P < 0·005$

Each value is a mean ± S.E.M.; n is the number of experiments.

Ratios of adrenaline to noradrenaline in the perfusates of peptide-stimulated adrenals

As shown in Fig. 2, perfusion with VIP (10 μM for 4 min) led to secretion of 27 ± 3 ng of catecholamines. The ratio of adrenaline to noradrenaline was 9·7 ± 0·8 ($n = 14$). A higher concentration of VIP (20 μM for 4 min) led to secretion of greater amounts of catecholamines (102 ± 12 ng) but the ratio of adrenaline to noradrenaline was not significantly different from that at the lower concentration (10·9 ± 1·5, $n = 6$).

A concentration of PACAP as low as 0·01 μM produced a significant increase in the secretion of catecholamines and the secretion increased with an increase in the concentration of PACAP (Fig. 2*A*). The ratios of adrenaline to noradrenaline was determined at all these concentrations and ranged from 6·2 to 6·8 (Fig. 2*B*); the differences were statistically not significant. These ratios were, however, significantly different from the ratios of adrenaline to noradrenaline evoked by VIP and ACh ($P < 0·001$; Table 2). PACAP or VIP obtained from different sources (see Methods) had similar potency in stimulating the secretion of catecholamines.

Ratios of adrenaline to noradrenaline in perfusates after stimulation of splanchnic nerves at different frequencies

Peptidergic and cholinergic transmitters are released in different proportions at different frequencies of splanchnic

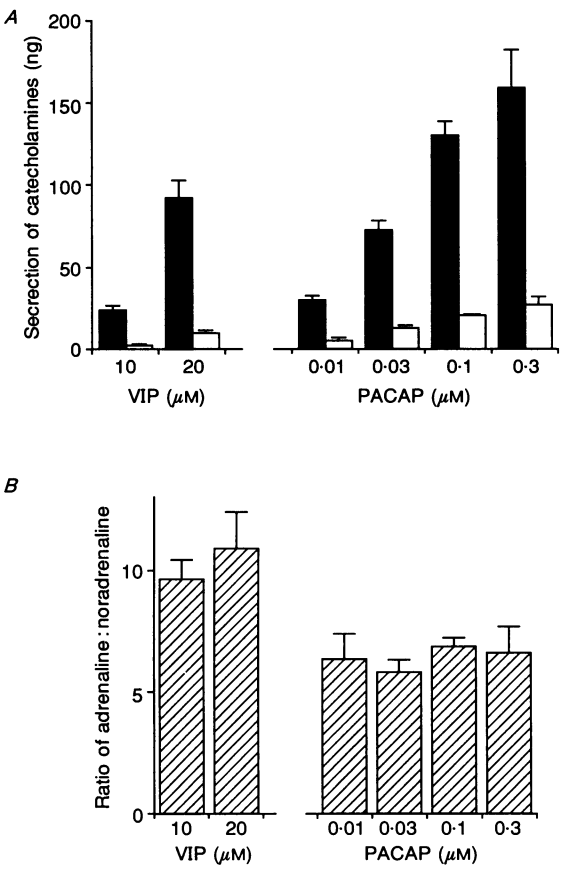

Figure 2. Secretion of catecholamines induced by different concentrations of VIP and PACAP
The intervals between successive exposures to PACAP varied between 30 and 60 min to allow the secretory responses to return to basal level. Each column represents a mean of 5–22 experiments. VIP and PACAP were tested in different glands. Vertical lines show S.E.M. *A*, adrenaline (filled columns); noradrenaline (open columns). *B*, ratios of adrenaline to noradrenaline (hatched columns).

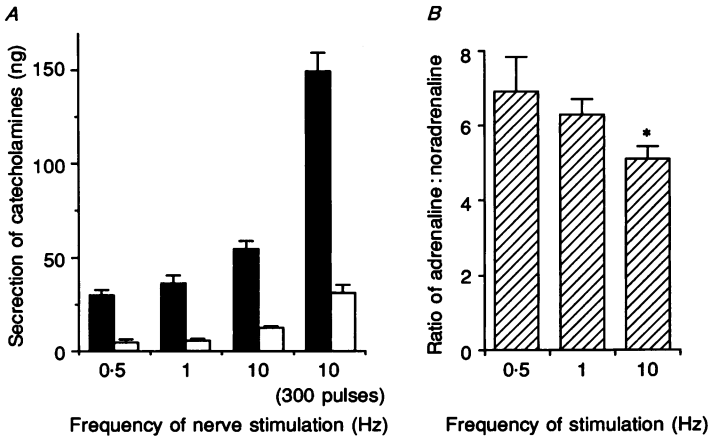

Figure 3. Secretion of catecholamines induced by field stimulation
Stimulations were given by delivering 150 pulses at 0·5, 1 and 10 Hz, or 300 pulses at 10 Hz, as indicated. Perfusates were collected for 8 min at 0·5 Hz stimulation and 4 min for the remaining stimulations. The same adrenal gland was used for all the stimulation frequencies. The interval between stimulations was 15 min. Each column represents a mean of 5–20 experiments. Vertical lines show S.E.M. *A*, adrenaline (filled columns); noradrenaline (open columns). *B*, ratios of adrenaline to noradrenaline (hatched columns). $*P < 0·025$ compared to 1 Hz.

nerve stimulation. VIP-like material is released in greater proportion to ACh at lower frequencies whereas ACh predominates at higher frequencies (Wakade *et al.* 1991). Since ACh and VIP stimulate secretion of the catecholamines in different proportions as shown in Figs 1 and 2, it was expected that electrical stimulation of splanchnic nerves at different frequencies should yield different proportions of catecholamines in the perfusate. As shown in Fig. 3, stimulation at 0·5 Hz for 5 min (150 pulses) released $30 \pm 2·7$ ng adrenaline and $5·2 \pm 0·86$ ng noradrenaline. The ratio of adrenaline to noradrenaline was $6·9 \pm 0·96$ ($n = 7$). Stimulation at 1 Hz for 2 min 30 s released $36 \pm 4·6$ ng adrenaline and $5·8 \pm 0·6$ ng noradrenaline. The ratio of adrenaline to noradrenaline was $6·3 \pm 0·4$ ($n = 17$). The secretion increased to $54 \pm 3·8$ ng adrenaline and $12 \pm 1·1$ ng noradrenaline and the ratio was reduced to $5·1 \pm 0·3$ at 10 Hz for 15 s ($n = 20$). Longer stimulation at 10 Hz for 30 s (300 pulses) released $149 \pm 9·7$ ng adrenaline and $31 \pm 4·1$ ng noradrenaline. The ratio was $5·2 \pm 0·9$ ($n = 5$) and was not statistically different from the ratio at 10 Hz for 15 s but the ratios of adrenaline to noradrenaline at 1 and 10 Hz were significantly different ($P < 0·025$).

Comparison of catecholamine secretion evoked by continuous or burst stimulation

There is evidence that the output of catecholamines from the adrenal gland and the proportion of noradrenaline released were significantly enhanced by electrically stimulating the splanchnic nerves of conscious calves in bursts of higher frequency compared to the same number of pulses delivered continuously at lower frequency (Bloom, Edwards & Jones, 1988; Edwards & Jones, 1989). We compared 3 Hz continuous with 30 Hz burst stimulation on the secretion and the ratio of secreted catecholamines. Continuous stimulation at 3 Hz for 50 s led to secretion of $48 \pm 11·6$ ng adrenaline and $6·5 \pm 1·8$ ng noradrenaline. The ratio was $7·4 \pm 0·5$ ($n = 3$). Burst stimulation at 30 Hz for 1 s at 10 s intervals (total 150 pulses) led to the secretion of 29 ± 4 ng adrenaline and $6·1 \pm 1·6$ ng noradrenaline. The ratio was $5 \pm 0·4$ ($n = 5$). The two ratios were statistically different ($P < 0·02$; Fig. 4). However, the amounts and the ratios of catecholamines secreted in response to 30 Hz burst stimulation were almost identical to those secreted in response to 30 Hz continuous stimulation.

DISCUSSION

As summarized in Table 2, our studies show that the profiles of catecholamines secreted in response to ACh and VIP, the cholinergic and non-cholinergic transmitters of the rat adrenal medulla, were entirely different. ACh induced secretion of both adrenaline and noradrenaline and their ratio was somewhat similar to the ratio found in the adrenal medulla. On the other hand, secretion evoked by VIP had a ratio of adrenaline to noradrenaline of 10 : 1, indicating that

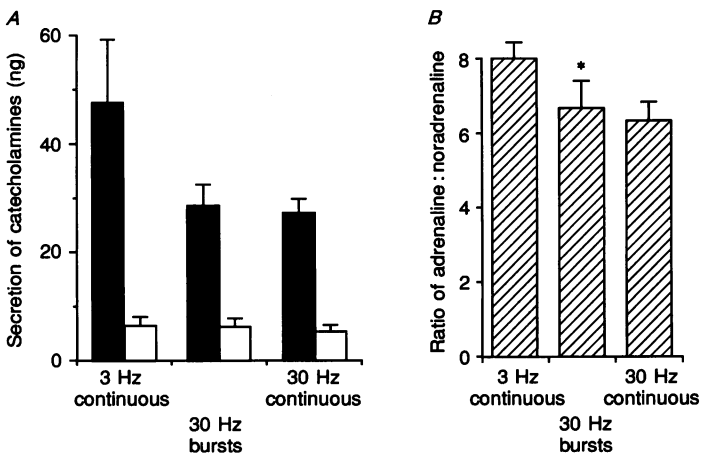

Figure 4. Comparison of secretion of catecholamines evoked by bursts *versus* continuous field stimulation
Continuous stimulation was given at 3 Hz for 50 s and at 30 Hz for 5 s. Burst stimulation was applied at 30 Hz for 1 s at 10 s intervals (total 150 pulses). Samples were collected over 4 min. The interval between stimulations was 15 min. The same adrenal gland was used for both types of stimulation. Each column represents a mean of 3–7 experiments. Vertical lines show S.E.M. *A*, adrenaline (filled columns); noradrenaline (open columns). *B*, ratios of adrenaline to noradrenaline (hatched columns). $*P < 0·02$ compared to 3 Hz.

VIP resulted in secretion mostly of adrenaline. The difference in this secretory pattern of ACh and VIP was not dependent on the degree of stimulation of chromaffin cells by these agents. For example, 10 μM ACh and 20 μM VIP produced comparable secretory responses yet the ratios differed significantly. These findings imply that the variations in the amounts of adrenaline and noradrenaline secreted by ACh and VIP may not depend on their potency but on their ability to stimulate adrenaline *versus* noradrenaline chromaffin cells specifically. As pointed out in the Introduction, there is ample evidence for two separate types of chromaffin cells in the rat adrenal medulla and they are differentially activated by different types of stressful condition and agonist. However, the mechanism of differential secretion of catecholamines by various agonists remains unknown. The present results raise some interesting possibilities for the differences observed in the secretory pattern by ACh and VIP. It is likely that adrenaline cells as compared to noradrenaline cells are rich in peptidergic receptors. Such a difference in the distribution of receptors could account for proportionally greater secretion of adrenaline by VIP. Another consideration is that coupling of second-messenger systems to membrane receptors could be different in adrenaline and noradrenaline cells. Currently, there is no information about receptor distribution for cholinergic *versus* peptidergic agonists in adrenaline- and noradrenaline-containing cells. Interestingly, Marley, Bunn, Wan, Allen & Mendelsohn (1989) have presented autoradiographic evidence for greater angiotensin II binding receptors on adrenaline than on noradrenaline chromaffin cells in bovine adrenal medulla. Differential secretion of noradrenaline and adrenaline with identical stimulation has been attributed to differences in the exocytotic mechanisms in noradrenaline and adrenaline chromaffin cells (Marley & Livett, 1987).

Among several substances tested for their stimulatory effect on chromaffin cells of the rat adrenal gland, PACAP was found to be the most potent substance. In comparison with ACh and VIP, PACAP was approximately 100 times more potent in evoking secretion of catecholamines. The reason for such high potency remains unknown at this time. Since increase in intracellular cAMP is known to facilitate the secretion of catecholamines in the rat adrenal medulla (Malhotra, Wakade & Wakade, 1989), it is likely that PACAP could markedly elevate cAMP levels in chromaffin cells to facilitate secretion. However, increase in cAMP alone is not sufficient to trigger secretion. A simultaneous increase in Ca^{2+} is necessary to initiate the secretory process (Malhotra *et al.* 1989). We do not know the exact mechanism of Ca^{2+} elevation by PACAP in the rat adrenal medulla. There is some evidence from cultured rat chromaffin cells that secretion of catecholamines induced by PACAP is associated with an increase in intracellular cAMP and Ca^{2+} (Watanabe *et al.* 1992).

Existence of PACAP immunoreactivity, together with specific PACAP receptors (see Introduction), and the high potency of the peptide in stimulating chromaffin cells (present study) suggest that PACAP could serve the function of a non-cholinergic transmitter in the rat adrenal medulla. Of course, this raises an important issue about whether PACAP and VIP are two separate non-cholinergic neurotransmitters or immunologically indistinguishable peptides. There is fairly good evidence for two types of receptor specific for PACAP and VIP in rat adrenal gland (Shivers *et al.* 1991). In support of these studies our data show that the ratios of catecholamines secreted in response to VIP and PACAP are significantly different. All these lines of evidence favour the idea that PACAP and VIP are two distinct neurotransmitters. However, more definitive data are needed to resolve this issue.

Differential secretion of adrenaline and noradrenaline in response to ACh and the peptides was used as an index to strengthen further our hypothesis that ACh and VIP are involved in catecholamine secretion at different levels of neuronal activity (Malhotra & Wakade, 1987; Wakade *et al.* 1991). If the secretion of catecholamines at basal levels is mostly under the control of peptides, and if the actions of endogenously released peptides are similar to those shown for exogenous VIP and PACAP, we would expect the ratios of adrenaline to noradrenaline at low frequencies of electrical stimulation to be closer to those secreted in response to the peptides. This is what we found. Between 0·5 and 3 Hz stimulation, the ratio was 6·6, in the range secreted in response to VIP and PACAP. At higher frequencies (10–30 Hz), the ratio shifted to 5·2, closer to the ratio secreted in response to ACh (Table 2). This variation in the amount and the ratio of secreted catecholamines at different frequencies of electrical stimulation lends additional support to our hypothesis of peptidergic dominance at low neuronal activity and cholinergic preponderance at higher neuronal activity. It is also clear from these data that if ACh was the only neurotransmitter released at all the frequencies of nerve stimulation, the ratios of adrenaline to noradrenaline would have remained unchanged. That was not the case.

We were unable to reproduce the effects of two modes of stimulation, as reported by Bloom, Edwards & Jones (1988) and Edwards & Jones (1989) in the adrenal glands of conscious calves. In our preparation, the amounts of catecholamines secreted at 30 Hz were less than those secreted at lower stimulation frequencies. The difference between the present study and the earlier work of other investigators may lie in the method of stimulation of splanchnic neurons. Transmural or field stimulation excites all the neuronal elements present in the adrenal medulla. Placing a bipolar electrode on splanchnic axons and using stimulation parameters of modest strength somewhat mimics physiological stimulation of the adrenal medulla. Another important difference is the nature of these two preparations. We used isolated rat adrenal gland retrogradely perfused with physiological salt solution whereas others used adrenals of conscious calves pretreated with naloxone with intact blood circulation. In any event, the main point of our study is that the ratio of adrenaline to noradrenaline secreted at 3 Hz is in the range of those found in response to peptidergic transmitters, whereas the ratios secreted at 30 Hz remain near those found in response to ACh regardless of the stimulation protocol.

In conclusion, differential secretion of VIP and ACh at different levels of neuronal activity followed by preferential stimulation of adrenergic and noradrenergic chromaffin cells by these transmitters could have an important physiological function. Release of VIP (PACAP) under resting conditions would lead to secretion mostly of adrenaline to support a low level of metabolic function. However, when splanchnic nerve activity increases, as during stress and exercise, release of ACh will stimulate the secretion of noradrenaline to meet the higher metabolic needs created by stressful conditions.

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