Release of 5-hydroxytryptamine, dopamine and noradrenaline in the rat vas deferens in the presence of compound 48/80, veratridine or K^+

¹Stella Maris Celuch & B. Duff Sloley

Neuropsychiatric Research Unit, Cancer and Medical Research Building, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

1 The release of 5-hydroxytryptamine (5-HT), dopamine and noradrenaline (NA) from the rat isolated vas deferens was estimated by high performance liquid chromatography with electrochemical detection.

2 Compound 48/80, which is known to release 5-HT and histamine from mast cells, induced a concentration-related, rapid overflow of 5-HT from the rat isolated vas deferens, releasing about 50% of the 5-HT present in the tissue at a concentration of $300 \mu\text{g ml}^{-1}$. The drug did not induce the release of either dopamine or NA.

3 The depolarizing agents veratridine ($10 \mu\text{M}$) or K^+ (100 mM) induced the release of 5–10% of the 5-HT present in the vas deferens, but did not evoke the overflow of 5-HT from isolated mast cells.

4 Veratridine induced the release of a greater proportion of the dopamine than of the NA contained in the vas deferens (41.0 ± 5.5 vs $17.9 \pm 2.0\%$, after 20 min of exposure to the drug, $n = 6$). In contrast, K^+ evoked the release of both amines equally.

5 It is concluded that in the rat vas deferens 5-HT is stored in mast cells and also in cells responsive to depolarizing stimuli. It is suggested that 5-HT could be a neuromodulator or a neurotransmitter in this organ. Veratridine and K^+ induce the release of dopamine in addition to NA.

Introduction

The rat vas deferens contains 5-hydroxytryptamine (5-HT) and it has been observed that the tissue concentrations of the amine are higher in regions nearer the prostatic gland than the epididymis (Celuch & Sloley, 1988). Moreover, 5-HT seems to be synthesized and deaminated in the rat vas deferens (Fuenmayor *et al.*, 1976; Celuch & Sloley, 1988). The precise location of 5-HT in the rat vas deferens is not known, although it has been suggested that the amine could be stored in mast cells (Fuenmayor *et al.*, 1976). We have examined the ability of compound 48/80 (a stimulant of mast cell secretion), or depolarizing agents (veratridine or K^+) to release 5-HT from the isolated rat vas deferens. The release of dopamine and noradrenaline (NA) by the above mentioned stimuli was also analysed.

Methods

Rat isolated vas deferens

Male Wistar rats of 200–230 g body weight were killed by a blow to the head and exsanguinated. The

vasa deferentia were isolated, cleaned of surrounding fat and of loose connective tissue and opened by a longitudinal cut. The blood contained in the main arteries was removed as much as possible by gently squeezing the vas deferens with the fingers while the organ was immersed in Krebs solution. Tissues were attached to a polypropylene cannula which also carried the gassing line and incubated for 30 min in glass test tubes containing 1 ml of Krebs solution at 37°C , bubbled with 95% O_2 plus 5% CO_2 , containing pargyline ($10 \mu\text{M}$) to inhibit monoamine oxidase activity. During this period the vas deferens was transferred to fresh incubation medium every 10 min. A further incubation (5–20 min duration) was performed using 0.5 ml Krebs solution containing compound 48/80, veratridine ($10 \mu\text{M}$) or K^+ (100 mM). Control experiments were performed by incubating the tissues in Krebs solution with $4.7 \mu\text{M}$ K^+ . The composition of the Krebs solution was (mM): NaCl 118.0, KCl 4.7, CaCl_2 2.6, MgCl_2 1.2, NaHCO_3 25.0, NaH_2PO_4 1.0, glucose 11.1, EDTA 0.004, ascorbic acid 0.055. The K^+ -enriched Krebs solution contained 100 mM KCl and 22.6 mM NaCl.

At the end of the experiment the tissues were

¹ Author for correspondence.

blotted dry, frozen immediately on dry ice and stored in polypropylene microtubes at -70°C until analysis (1–2 days). Six hundred μl of 0.2 N perchloric acid containing 0.1 mM sodium metabisulphite, 0.25 mM EDTA and $0.1 \mu\text{g ml}^{-1}$ isoprenaline as internal standard were added to each microtube. The samples were homogenized by ultrasonic disruption for 15 s using a Brausonics 1510 ultrasonic probe (400 W setting) and centrifuged for 15 min in an Eppendorf 5412 desk top centrifuge. Aliquots of the supernatants were injected into a high performance liquid chromatograph (h.p.l.c.) equipped with electrochemical detection for determination of 5-HT, dopamine and NA.

To determine the amounts of 5-HT, dopamine and NA released into the incubation medium, the Krebs solution (0.5 ml) corresponding to the last period of incubation was mixed with 100 μl of the 0.2 N perchloric acid solution and centrifuged. Aliquots of the supernatant (100 μl) were injected directly onto the h.p.l.c. column. Chromatograph separations were performed as previously described (Yu *et al.*, 1986) at room temperature on a $250 \times 4.6 \text{ mm i.d.}$ Ultrasphere I.P. analytical column packed with C-18 spherical-5 μm particles (Beckman, Toronto, Ont.). An SSI precolumn filter (0.5 μm filter elements, Terochem, Rexdale, Ont.) and $30 \times 4.6 \text{ mm i.d.}$ Brownlee MPLCRP-18 SPHERI-5 guard column (Technical Marketing Associates, Calgary, Alberta) were installed between the Waters WISP 710B automated sample injector and the analytical column. The mobile phase, comprising 75 mM monobasic sodium phosphate, 1 mM sodium octyl sulphate, 500 μM EDTA, 12.5% acetonitrile, with the final pH adjusted to 2.75 with phosphoric acid, was pumped through the column at 1.0 ml min^{-1} using a Waters M45 solvent delivery system (Millipore, Mississauga, Ont.). Before the addition of acetonitrile, the buffer was filtered through a 0.22 μm Millipore filter. Degassing of the mobile phase was accomplished by vacuum. The detector consisted of a BAS Model TL-5A thin-layer amperometric electrode (Mandel, Rockwood, Ont.) which was controlled at 0.93 V vs a Ag-AgCl reference electrode. Signals for the detector were integrated by peak area using a Spectra-Physics SP-4290 integrator (San José, CA).

Rat isolated peritoneal mast cells

Four male Wistar rats (280–320 g body weight) received pargyline 200 mg kg^{-1} (i.p.) and were killed 2 h later by cervical dislocation. Eight ml of 'washing saline solution' were injected intraperitoneally and the abdomen gently massaged for 2 min. The abdominal fluid was collected in ice-cooled polypropylene tubes and centrifuged at 400 g for 5 min. The pellet was resuspended in 'washing saline solu-

tion', divided into two parts and the centrifugation repeated. One of the resultant pellets was suspended in 6 ml ice-cold 'incubation saline solution' with normal K^{+} concentration (2.7 mM) and the other was suspended in 6 ml of K^{+} -enriched 'incubation saline solution' (K^{+} 100 mM). One ml aliquots of the cell suspensions were incubated at 37°C for 15 min. Veratridine (10 μM) or compound 48/80 ($1 \mu\text{g ml}^{-1}$) was added for the last 10 min of incubation to two of the tubes containing normal K^{+} -concentration 'incubation saline' while a third one served as control. At the end of the incubation period the samples were cooled in ice and centrifuged at 1000 g for 5 min. The pellets and supernatants were processed for determination of 5-HT by h.p.l.c. as previously described for the vas deferens.

The 'washing saline solution' contained (mM): NaCl 137, KCl 2.7, NaH_2PO_4 0.4, HEPES 10, glucose 5.5, bovine serum albumin 0.05%. The 'incubation saline solution' contained, in addition, 1 mM CaCl_2 and 0.5 mM MgCl_2 (Lohse *et al.*, 1987). When the 'incubation saline solution' contained 100 mM KCl, the concentration of NaCl was lowered to 39.7 mM. All saline solutions contained 0.055 mM ascorbic acid and 0.004 mM EDTA.

Fractional release (%)

The overflow of amines induced by stimulating agents or released spontaneously is expressed as a percentage of the calculated amine content of the vas deferens or peritoneal mast cells at the beginning of the period of incubation. Fractional release (%) = $100 \times \text{amine released to the incubation medium} \div \text{amine released} + \text{amine left in the tissue or in the cells at the end of the incubation period}$.

Drugs

Compound 48/80 (condensation product of N-methyl-p-methoxyphenethylamine with formaldehyde), pargyline hydrochloride and veratridine were purchased from Sigma (St. Louis, MO, U.S.A.). Chemicals for h.p.l.c. with the exception of acetonitrile (h.p.l.c. grade, Caledon, Georgetown, Ontario, Canada) and octyl sodium sulphate (Eastman Kodak, Rochester, NY, U.S.A.) were obtained from Sigma (St. Louis, MO, U.S.A.) or were laboratory grade and were used without further purification.

Statistics

Data are expressed as mean values $\pm$ s.e.mean. Differences between means were examined by one factor analysis of variance on logarithm transformed

data, followed by Dunnett's test (Sokal & Rohlf, 1981).

Results

The amine concentrations in rat vas deferens before adding stimulating agents to the incubation medium were calculated to be ($n = 132$): 5-HT $162.2 \pm 7.8 \text{ ng g}^{-1}$; dopamine $293.5 \pm 12.5 \text{ ng g}^{-1}$; NA $14.16 \pm 0.19 \mu\text{g g}^{-1}$ and tissue weight was $38.76 \pm 0.72 \text{ mg}$.

Compound 48/80 ($1\text{--}300 \mu\text{g ml}^{-1}$) induced a concentration-dependent release of 5-HT in the rat isolated vas deferens. At the highest concentration used, the drug released about 50% of the amine present in the tissue (Figure 1). The overflow of 5-HT induced by compound 48/80 ($10 \mu\text{g ml}^{-1}$) appeared to be complete after 2 min of exposure of the tissue to the drug, but the releasing effect was observed even at 30 s (Figure 2a). Compound 48/80 did not induce release of NA or dopamine.

Veratridine ($10 \mu\text{M}$) or K^+ (100 mM) also produced a significant increase in 5-HT overflow. The maximal fractional release (about 10%) was observed after 15–20 min of incubation with either depolarizing agent (Figure 2a). Both veratridine and K^+ induced the release of dopamine and NA. The fractional release of dopamine and NA induced by K^+ were similar (Figure 2b,c). The only exception was that after 20 min of incubation with K^+ -enriched medium, the fractional release of dopamine

Figure 1 Release of 5-hydroxytryptamine (5-HT) by compound 48/80 in the rat isolated vas deferens. The overflow of 5-HT is expressed as a percentage of the amount of amine present in the tissue at the beginning of the incubation period (5 min). Shown are mean values ($n = 4$) and vertical lines indicate s.e.mean.

Figure 2 Time-course for the release of (a) 5-hydroxytryptamine (5-HT), (b) noradrenaline (NA) and (c) dopamine (DA), induced by compound 48/80 ($10 \mu\text{g ml}^{-1}$, ●), veratridine ($10 \mu\text{M}$, ■) and potassium (100 mM , ◆). The overflow of amine to the incubation medium is expressed as a percentage of the amount of amine present in the tissue. (▲) Spontaneous fractional release (%) in the absence of stimulating agents. As dopamine and noradrenaline were not released by compound 48/80, these results have been omitted for clarity. Shown are mean values of 6 determinations and vertical lines indicate s.e.mean. * $P < 0.01$ compared to the spontaneous release at the corresponding period of incubation (Dunnett's test). The test was not performed for dopamine values as the spontaneous release of the amine was below the limit of sensitivity of detection.

($8.9 \pm 2.3\%$) was slightly higher than that of NA ($4.3 \pm 0.6\%$) ($n = 6$). In contrast, veratridine released 2–3 times more dopamine than NA in terms of fractional release at all periods of incubation tested (Figure 2b,c). Dopamine was not detected in the incubation medium in the absence of stimulating agents. The spontaneous fractional release of NA ranged from $0.08 \pm 0.04\%$ (5 min, $n = 6$) to $0.23 \pm 0.08\%$ (20 min, $n = 6$).

To investigate whether veratridine or K^+ induced the release of 5-HT from mast cells, isolated peritoneal mast cells were exposed to the depolarizing agents for 10–15 min. Neither veratridine ($10 \mu M$) nor K^+ (100 mM) induced the secretion of 5-HT, whereas compound 48/80 ($1 \mu g ml^{-1}$) produced a significant 80% depletion of the amine in mast cells.

Discussion

Compound 48/80, which is known to induce the release of 5-HT and histamine from mast cells (Moran *et al.*, 1962; Irman-Florjanc & Erjavec, 1983; Koibuchi *et al.*, 1985), caused a rapid and pronounced overflow of 5-HT from the rat isolated vas deferens (Figures 1 and 2). This finding indicates that a considerable part of the amine in this organ is stored in mast cells, as previously suggested by Fuenmayor *et al.* (1976). Compound 48/80 is, however, less effective in the vas deferens than in isolated mast cells. At $1 \mu g ml^{-1}$, compound 48/80 produces an almost complete depletion of both histamine and 5-HT in isolated mast cells (Carraway *et al.*, 1984; this study), but a concentration 300 times higher released only 50% of the 5-HT content of the rat vas deferens (Figure 2).

Mast cells are not the only source of 5-HT in the rat vas deferens since veratridine and a high concentration of K^+ released 5-hydroxytryptamine from this tissue (Figure 2), but were ineffective in isolated peritoneal mast cells. Contamination with 5-HT from blood platelets is considered unlikely because the volume of blood remaining in the vas deferens was very small. Moreover, the releasing effect of veratridine was still observed in one vas deferens where the blood had been washed out by perfusion with saline (results not shown).

Some possible sources for the 5-HT which was released by either veratridine or K^+ could include 5-hydroxytryptaminergic neurones, chromaffin cells or small intensely fluorescent (SIF) cells. 5-Hydroxytryptaminergic neurones and enterochromaffin cells containing 5-HT are known to exist in the gut (Benditt & Wong, 1957; Penttila, 1966; Robinson & Gershon, 1971; Juorio & Gabella, 1974; Gershon & Sherman, 1982; Holzer & Sko-

fitsch, 1984), and cells emitting a yellow indole fluorescence, morphologically resembling enterochromaffin cells, were observed in human prostatic mucosa (Baumgarten *et al.*, 1968). SIF cells possessing 5-HT have been identified in the superior cervical ganglion of the rat (Eranko & Harkonen, 1965; Verhofstad *et al.*, 1981). Whether the rat vas deferens possesses one or more of these kinds of 5-HT-containing cells is uncertain. An early cytochemical study did not detect endogenous 5-HT in the rat vas deferens (Jaim-Etcheverry & Zieher, 1969), but further investigation is required since the amine has been detected fluorometrically (Fuenmayor *et al.*, 1976) and by h.p.l.c. (present study).

The finding that depolarizing agents release 5-HT suggests that the amine may function as a neurotransmitter or a neuromodulator in the rat vas deferens. This suggestion is supported by pharmacological studies which show that 5-HT induces stimulative and inhibitory effects, mediated by specific 5-HT receptors, on smooth muscle contractility of the rat vas deferens (Kapur & Mottram, 1979; Hay & Wadsworth, 1982; Moritoki *et al.*, 1986a,b). According to our information, however, this is the first study where the release of 5-HT from the rat vas deferens has been determined. It will be of interest to investigate if transmural nerve stimulation induces release of 5-HT as well as veratridine or K^+ .

For many years it was thought that the main role of dopamine in sympathetic nerves was as a precursor of NA. However, there is now evidence that sympathetic nerve endings as well as smooth muscle possess dopamine receptors (Enero & Langer, 1975; Simon & Van Maanen, 1976; Tayo, 1979; Ohlstein & Berkowitz, 1985). In addition, the release of dopamine together with NA has been detected in several experimental conditions (Soares-da-Silva, 1987; see also review by Bell, 1988). These findings suggest that dopamine has a further role in sympathetic neurotransmission. In the rat isolated vas deferens veratridine induced the release of a larger proportion of the dopamine than of the NA contained in the tissue. In contrast, depolarization by K^+ induced the release of both amines equally. A tentative explanation is that veratridine and K^+ induced a distinct mobilization of NA-storage vesicles containing different concentrations of dopamine (different proportions of small vs large dense cored vesicles?). According to Klein & Lagercrantz (1981, 1982), the high-affinity uptake of dopamine and the synthesis of NA do not occur in the small dense cored vesicles of sympathetic nerves, but in the large ones. Therefore, it is possible that the content of dopamine in both kinds of vesicles is different. The difference between veratridine and K^+ with respect to the

fractional release of dopamine and NA in the rat vas deferens could be a consequence of the distinct ionic movements across the cell membrane, caused by either depolarizing agent (Ito *et al.*, 1979; Adler-Graschinsky *et al.*, 1984). The preferential release of dopamine over NA, induced by veratridine (this study) resembles the overflow of these amines during transmural nerve stimulation of the rat and guinea-pig vas deferens (Bell *et al.*, 1984).

In conclusion, the present study shows that in the rat vas deferens, 5-HT is probably stored in mast cells and also in cells responsive to depolarizing stimuli (veratridine or K^+). It is suggested that 5-HT may be a neurotransmitter or a neuromodulator in

this organ. Veratridine induces the release of a larger proportion of the dopamine than of the NA present in the tissue, while K^+ induces the release of both amines equally.

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