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Release of vasoactive intestinal peptide from rat jejuno-ileum in vitro. Effect of various depolarizing agents[1]

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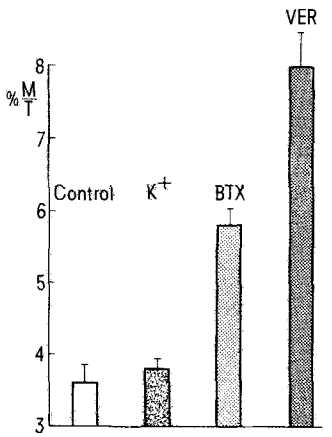
Summary. Vasoactive intestinal peptide (VIP) can be released in vitro from intestinal slices under veratridine and batrachotoxin depolarization, whereas potassium depolarization has no effect. The lack of an effect of potassium observed in this peripheral preparation is different from the positive action described for it in the CNS. The present data suggest that VIP can be released through different mechanisms in the peripheral and central nervous system.

Vasoactive intestinal peptide (VIP), which was first discovered in the gut[2], has also been found in the CNS[3,4]. In the CNS, we have recently shown that depolarizing agents such as potassium, veratridine and batrachotoxin can induce in vitro release of VIP from brain slices[5]. The response to depolarizing stimuli has been considered as one of the criteria to establish a substance as a neurotransmitter. The aim of the present work was to investigate whether those various depolarizing agents were able to induce the release of VIP from intestinal slices in vitro.

Materials and methods. Wistar male rats (250–300 g) were killed by decapitation. The jejuno-ileum was dissected out and washed several times with NaCl 0.24 M EDTA 2.5 mM pH 7.4 in order to remove the epithelial cells, as previously described[6]. The lamina propria was then scraped with a polyethylene tip and cross cut into slices (250 µm) with a McIlwain tissue chopper. Histological controls were performed at each step of the preparation. Microscopic observations showed that there were no more epithelial cells and that the muscular layers were not touched after scraping. The preparation only consisted of the lamina propria, which has been shown to contain VIP nerve terminals[7]. Slices were washed several times until a clear supernatant was obtained in cold Krebs Ringer Bicarbonate (KRB) buffer (pH. 7.4) containing (mM): NaCl 118, KCl 5, $CaCl_2$ 2.5; KH_2PO_4 1.2, $NaHCO_3$ 25, Glucose 10, Bacitracin 2×10^{-5} M and trasylol 500 kIU/ml. Slices were preincubated in KRB buffer under 95% O_2 - 5% CO_2 during 30 min at 37 °C. The incubation was started by addition of a lamina propria preparation (5 mg tissue in 200 µl KRB) to polyethylene tubes containing the various substances being tested. The incubation was then carried out for 10 min at 37 °C and ended by centrifugation in an Eppendorf microcentrifuge at 10,000 × g for 2 min. The slices were immediately extracted with 0.1 N HCl, sonicated for 15 sec and samples were frozen until assay. VIP content was assayed both in the supernatant and the tissue extract using a specific radioimmunoassay for the peptide[8]. VIP release was expressed as the percentage of VIP secreted in the medium over VIP content in the tissue.

Results and discussion. The effect of potassium (K^+), batrachotoxin (BTX) and veratridine (VER) on the release of VIP from the lamina propria of intestinal villi is shown on the figure. The basal amount of VIP released in the medium represents 3.6 ± 0.3% of the total content in the tissue. BTX (1 µM) and VER (50 µM) induced a significant release of VIP ($p < 0.01$ vs control) which represented 1.6- and 2.2-fold increases of the basal level, respectively. On the contrary, K^+ (56 mM) does not significantly increase the release of VIP from the intestinal preparation.

Increasing extracellular K^+ concentration in the incubation medium does not seem to influence the basal release of VIP from slices of the lamina propria. This lack of effect is not due to the absence of VIP nerve terminals in this region, since both VER and BTX increase the secretion of the peptide under similar conditions. Moreover, immunocytochemical data have shown abundant VIP-containing cell bodies and fibers in the lamina propria of different species



Effect of potassium 56 mM (K^+), batrachotoxin 1 µM (BTX) and veratridine 50 µM (VER) on the release of VIP from gut lamina propria. The release of VIP is expressed as the percentage of VIP secreted in the medium (M) over the amount of VIP present in the tissue (T). The data are mean ± SEM of 8 determinations and are a representative example of 6 different experiments. Data from the other experiments are similar, and show no significant differences between groups, as found by analysis of variance.

in close connection with epithelial cells[7]. The present results suggest that VIP immunoreactive fibers in the gut lamina propria are sensitive to membrane depolarization induced by VER and BTX and not sensitive to K^+ depolarization. This is different from what has been reported for the release of various neuropeptides in the CNS. In the case of VIP for instance, K^+, VER and BTX have been shown to induce the release of VIP from nerve endings of the cortex and amygdala[5]. The present data suggest that the release of a neuropeptide such as VIP, found both in the CNS and in the gut may by regulated by different mechanisms possibly involving various ionic channels. In the gut, it seems that VIP release is more sensitive to sodium than calcium ions since the effects of VER and BTX are known to act through sodium channels[5,9], whereas potassium involves calcium-dependent channels[5,10,11]. Further experiments are necessary to test whether VIP can be released under different physiological conditions in the CNS and in the gut.

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Location of dopamine stores in rat kidney[1]

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Summary. In normotensive and genetically hypertensive Wistar rats, chronic renal denervation reduces renal cortical levels of noradrenaline and dopamine by more than 90%. Non-neural stores of renal dopamine are therefore small or absent.

Dopamine (DA) is known to increase renal blood flow and to produce natriuresis, through actions on intrarenal DA receptors[2], and there is currently considerable interest in the possibility that endogenous DA of renal origin may play a physiological role in the kidney[3-5].

A major difficulty in determining the status of DA as a regulator of renal function is the discrimination between DA released from intrarenal sites and DA synthesized or deconjugated from plasma sources during its passage through the kidney. One prerequisite for such distinction is knowing the quantity and location of intrarenal DA stores. The present investigation is concerned with determination of whether, in the rat, non-neuronal stores of DA exist in the renal cortex. In view of recent proposals that renal activity of catecholamines may be implicated in the development of high blood pressure in rats with genetic hypertension[6-9] the results have been compared for a strain of normotensive and a closely related strain of genetically hypertensive animals.

Materials and methods. The rats used were F8-F9 adult male descendants of breeding stock obtained from the Otago Medical School, and which represented pure Wistar lines of normotensive and genetically related spontaneously hypertensive animals[10]. Both strains were maintained by sibling matings within each generation. They were fed a proprietary rat chow diet (quoted Na content 0.5%) and allowed access to water ad libitum.

Renal denervation was accomplished via a flank incision under sodium pentobarbitone anesthesia (30 mg/kg i.p.) by swabbing the left renal artery and vein with 3% phenol in absolute alcohol. Antibiotic powder was dusted into the abdominal cavity, and the incision was closed with muscle sutures and skin Michel clips. 6-7 days was allowed after operation for full neural degeneration to occur. No histological examinations were performed, but on macroscopic inspection both the denervated and the contralateral control kidneys appeared similar in size, texture and vascularity.

Kidneys were removed from animals killed by cervical dislocation, and the cortical tissue was quickly dissected away from the medulla and chilled. After blotting on filter paper, the samples were weighed, minced and placed in ice-cold 0.1 M perchloric acid containing 3×10^{-5} M EDTA, 10^{-4} M sodium metabisulphite and α-methyl dopamine as internal standard. Catecholamines were extracted with alumina, separated using high pressure liquid chromatography on cation-exchange resin and assayed by oxidative electrochemistry. The details of these procedures and the sensitivity and reproducibility of the assay have been reported previously[11,12].

Results and discussion. Renal cortical contents of catecholamines in normotensive rats were: NA 148 ± 13 ng/g (mean $\pm$ SEM), DA 7.6 ± 2.0 ng/g ($n = 14$), values which were very similar to those reported previously for a Sprague-Dawley strain[11]. Adrenaline was usually below the limits of detectibility (100-150 pg/g).

In renal cortices of a series of genetically hypertensive rats, contents were NA 177 ± 10 ng/g, DA 7.0 ± 2.9 ng/g ($n = 10$). Neither the absolute concentration of amines or the ratio between them were significantly different when normotensive and hypertensive populations were compared using a 2-tailed Student's t-test ($0.05 > p < 0.1$). Several recent studies have reported that renal denervation delays the development of high blood pressure in both the Kyoto and the Otago strains of genetically hypertensive rat[6-8], while a further study has presented evidence for increased numbers