The effect of inorganic salts on renal tissue dopamine levels in the rat

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Increasing dietary sodium chloride in the rat results in an increased urinary excretion of free dopamine (Ball & Lee, 1977a). Further work has demonstrated that the administration of the chlorides of potassium and ammonium also raises the urinary free dopamine, whereas giving sodium bicarbonate results in a fall (Ball, Oates & Lee, unpublished observations). The urinary changes in free dopamine (a weak base) could be influenced by the pH of the urine. We have, therefore, examined the changes in free dopamine in the kidney, brain and liver of rats given the chlorides of sodium, potassium and ammonium; and sodium bicarbonate.

Male Wistar rats (200–250 g) housed in groups of six were given either a low sodium diet (BP Nutrition ref. 821511) or the same food supplemented by 0.85 mmol of sodium, potassium or ammonium, as their chlorides, per g of food; or 0.85 mmol of sodium bicarbonate per g of food. Both the control and test groups of rats received the diet for four days, after which they were killed.

Kidney, liver and brain from each rat were homogenized in 5 volumes of ice-cold 0.155 M KCl. After deproteinization with perchloric acid the tissue-free dopamine was measured by the radiokinetic method of da Prada & Zürcher (1976). The recovery of dopamine added to tissue homogenates by this method was 55.3 ± 3.2% (mean ± s.e. mean).

Kidney tissue dopamine in the NaCl group was 3.51 ± 0.10 pmol/mg protein (mean ± s.e. mean) compared with its low sodium control group of 3.06 ± 0.12 pmol/mg protein ($t = 2.98$; $P < 0.02$); in the KCl group was 3.56 ± 0.08 pmol/mg protein compared with its control of 3.22 ± 0.08 pmol/mg protein ($t = 2.80$; $P < 0.02$); in the NH_4Cl group was 3.33 ± 0.20 pmol/mg protein compared with its control of 2.78 ± 0.11 pmol/mg protein ($t = 2.84$; $P < 0.02$). There was no change in the $NaHCO_3$ group (3.73 ± 0.12 pmol/mg protein) compared with its control of 3.63 ± 0.11 pmol/mg protein ($t = 0.61$; $P > 0.1$). No significant changes in the liver or brain free dopamine were found in any of the groups of rats ($P > 0.1$).

The changes in renal dopamine reflect the changes we have found in the urinary dopamine of rats treated similarly. The absence of a change in hepatic or cerebral dopamine levels suggests that the increments in renal dopamine are specific to this tissue. The simple hypothesis that intrarenal free dopamine is a factor in the control of sodium excretion (Ball & Lee, 1977b; Faucheaux, Buu & Kuchel, 1977) must now take account of the effects of potassium and ammonium chlorides, and sodium bicarbonate. The changes in intrarenal dopamine may represent an integrated response to the combined stimuli of osmolarity and hydrogen ion concentration.

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The effects of some neurotransmitter substances on the production of corticotrophin releasing factor by the rat hypothalamus *in vitro*

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The hypothalamus of the rat is viable *in vitro* (Bradbury, Burden, Hillhouse & Jones, 1974) and responds to certain neurotransmitter substances with changes in the level of its functional activity. We have used this preparation to study the production of corticotrophin releasing factor (CRF). Hypothalami were removed from rats and incubated as previously described by Buckingham & Hodges (1977a) in the presence and absence of various neurotransmitter substances and drugs which mimic or antagonize their actions. The CRF content of the hypothalami and the media in which they were incubated were determined using the sensitive and precise method which depends on the ability of the hypothalamic hormone to stimulate corticotrophin production by

adenohypophysial tissue *in vitro* (Buckingham & Hodges, 1977b). Acetylcholine (10^{-12}–10^{-9} M) and 5-hydroxytryptamine (5-HT) (10^{-9}–10^{-6} M) caused dose-related increases in CRF synthesis and release and their effects were antagonized by atropine, (1.4×10^{-11} M) hexamethonium (10^{-9} M) and cyproheptadine (10^{-7} M) and by methysergide (5×10^{-7} M) and cyproheptadine (10^{-7} M) respectively. Noradrenaline (10^{-8} M) also reduced the responses to acetylcholine and 5-HT. The actions of noradrenaline were mimicked by adrenaline (10^{-7} M), phenylephrine (10^{-8} M) and methoxamine (10^{-8} M) but not by isoprenaline (10^{-6} M) and antagonized by phentolamine (10^{-8} M) but not by atenolol (10^{-7} M). The results indicate the existence of cholinoceptors, 5-HT receptors and α-adrenoceptors in the hypothalamus, all of which may be involved in the control of the synthesis and release of the corticotrophin releasing factor.

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Evidence for a presynaptic inhibitory receptor for 5-hydroxytryptamine in dog isolated saphenous vein

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5-Hydroxytryptamine (5-HT) inhibits sympathetic neuronal activity in anaesthetized animals (Page & McCubbin, 1953). More recently it has been suggested that 5-HT has a presynaptic inhibitory action on neurones in the dorsal raphe nucleus (Farnebo & Hamberger, 1974; Haigler & Aghajanian, 1977). Since the dog isolated saphenous vein is a useful vascular preparation for studying presynaptic inhibitory agents (Vanhoutte & Shepherd, 1973; Verhaeghe, Vanhoutte & Shepherd, 1977) we have used it to examine the effects of 5-HT on contractile responses produced by electrical stimulation.

Dog saphenous vein strips were prepared as described previously (Apperley, Humphrey & Levy, 1977). The strips were mounted between platinum electrodes in Krebs solution at 37°C which contained indomethacin (2.8×10^{-6} mol/l) and cocaine (3.0×10^{-5} mol/l) to inhibit endogenous prostaglandin biosynthesis and uptake$_1$ respectively. The isometric contractions produced by electrical stimulation (0.1 ms, supramaximal voltage for 10 s) were frequency dependent (0.5–10 Hz). Stimulation at 2 Hz produced submaximal contractions of 0.75 ± 0.10 g (mean ± s.e. mean, $n = 20$). These contractions were almost completely blocked by tetrodotoxin (3.1×10^{-8} mol/l) or phentolamine (1.0×10^{-6} mol/l) but unaffected by mecamylamine (1.0×10^{-5} mol/l), suggesting that they were mediated predominantly via noradrenaline release from post-ganglionic neurones. Contractions of the saphenous vein induced by electrical stimulation were inhibited by 5-HT (1.0×10^{-9}–1.0×10^{-7} mol/l) in a concentration-dependent manner. The concentration of 5-HT which produced 50% inhibition was $3.2 \pm 0.6 \times 10^{-8}$ mol/l ($n = 20$); the maximal inhibition obtained was $67 \pm 4\%$. 5-HT slightly potentiated contractile responses to exogenous noradrenaline (1.0×10^{-8}–1.0×10^{-4} mol/l) which suggests that the site of the inhibitory action is presynaptic. The inhibitory effect of 5-HT was potentiated by cyproheptadine (1.0×10^{-6} mol/l) but was not antagonized by phentolamine (5.0×10^{-8} mol/l), propranolol (1.0×10^{-6} mol/l), morphine (1.0×10^{-5} mol/l), atropine (1.0×10^{-6} mol/l), mepyramine (1.0×10^{-6} mol/l) or cimetidine (1.0×10^{-5} mol/l). 5-HT also contracted the saphenous vein, although the threshold concentration was about 10 times higher than that required for inhibition of electrically induced contractions. These contractions to 5-HT were almost completely blocked by cyproheptadine (1.0×10^{-6} mol/l).

Our findings are in accord with a preliminary report that 5-HT inhibits electrically-induced release of tritiated noradrenaline from the dog saphenous vein (McGrath & Shepherd, 1976) and suggest that activation of an inhibitory presynaptic 5-HT receptor is involved. The receptor has yet to be fully characterized but, like the central inhibitory receptor (Haigler & Aghajanian, 1977), it is not blocked by the classical D-receptor antagonist cyproheptadine.