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PHYSIOLOGY

A Central Action of 5-Hydroxytryptamine and Noradrenaline

THE experiments reported here were based on the observation that an intra-carotid or intravenous injection of *d*-lysergic acid diethylamide (LSD-25) depresses the transmission of orthodromic impulses through the lateral geniculate nucleus of the cat[1]. The nature of this depression suggests that LSD-25 can compete with the excitatory transmitter for post-synaptic receptor sites. Consequently the known antagonism between LSD-25 and 5-hydroxytryptamine (5-HT) at receptors of smooth muscle[2] indicates the possibility that the as yet unknown central excitatory transmitter might be related to 5-HT. It is probable that the failure to demonstrate an action of 5-HT on these neurones[1] was due to the blood-brain barrier, which is relatively impermeable to this substance[3].

In the present investigation, 5-HT and a series of structurally similar compounds, including some catecholamines, have been applied electrophoretically to single neurones in the lateral geniculate nucleus of a cat anæsthetized with pentobarbital. The direct extracellular application of drugs circumvents the blood-brain barrier and the actions of four different compounds could be compared on single cells by the use of five-barrel electrodes[4]. The spike responses, 0·2–1·0 mV. in amplitude and of negative-positive voltage sequence, were recorded by means of the central barrel of the electrode, which contained 4 *M* sodium chloride. Most substances were passed as cations from saturated aqueous solutions of *p*H 3–4. These included 5-HT creatinine sulphate, tryptamine, bufotenine, LSD-25, 2-bromo-LSD(BOL-148), psilocin[5], noradrenaline bitartrate, adrenaline bitartrate and 3-hydroxytyramine. L-Glutamic acid and psilocybin[5] were applied as anions from solutions of the sodium salt at *p*H 8.

When an electrophoretic current was used to apply cations from a saturated aqueous solution of 5-HT creatinine sulphate, there was suppression of the orthodromic responses of neurones which responded monosynaptically, and occasionally repetitively, to an optic nerve volley. Such was not the case when the electrode contained creatinine sulphate instead of the 5-HT creatinine sulphate complex, hence it is reasonable to assume that the depression observed with the complex was due to 5-HT. The latency of this effect was reciprocally related to the magnitude of the electrophoretic current and for currents of 20–100 mμamp. was about 10–2 sec. Following the termination of the current flow the spike responses recovered in 5–20 sec. When L-glutamate was applied from another barrel, the excitation of the neurones, measured by the frequency of the firing evoked by the amino-acid[4], was unaltered by 5-HT. Furthermore, the antidromic spike responses of these neurones, evoked by stimulation of the optic radiation beneath the ipsilateral gyrus marginalis of the cerebral cortex, were unaffected by 5-HT. These actions of 5-HT are illustrated in Fig. 1.

All the substances mentioned here, except creatinine and L-glutamic acid, had actions identical with those of 5-HT. Although it is difficult to compare various substances quantitatively by these electrophoretic techniques[4], an approximate estimate of the potency of the compounds can be given. 5-HT was the most effective depressant of these cells, as

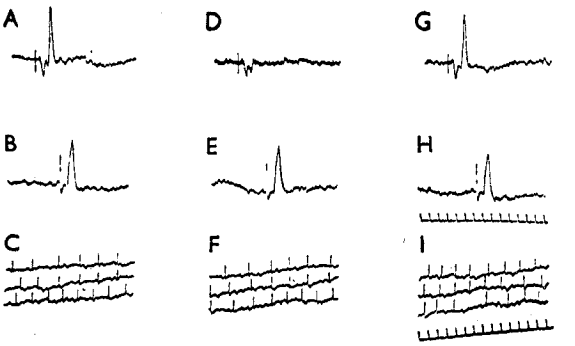

Fig. 1. Spike responses of a single neurone in the lateral geniculate nucleus responding monosynaptically to a contralateral optic nerve volley (*A*, *D*, *G*), antidromically to a stimulus to the ipsilateral optic radiation (*B*, *E*, *H*) and repetitively to L-glutamic acid (*C*, *F*, *I*) which was applied from one barrel of a five-barrel electrode using an anionic current of 100 mμamp. After the control responses *A* and *B*, a current of 20 mμamp. passed cations from an aqueous solution of 5-HT creatinine sulphate (*p*H 3·3) for 12 sec. The responses *D* and *E* were recorded 5–7 sec. after the current started and *G* and *H*, 5–7 sec. after it terminated. *C*, *F*, *I* were recorded with lower amplification (all spikes are approximately 0·5 mV. in amplitude) in another series: *C*, control; *F*, 31 sec. after a current of 20 mμamp. passed 5-HT creatinine sulphate into the environment of the cell. At this time orthodromic responses were suppressed. *I*, 2 min. after the 5-HT application ceased, the orthodromic responses having recovered fully. Time, msec. for *A*, *B*, *D*, *E*, *G*, *H* beneath *H*; 10 msec. for *C*, *F*, *I* beneath *I*

determined by the magnitude of the electrophoretic current necessary to block the excitation of single cells within a given time. The other agents were less effective, the descending order of potency being tryptamine; bufotenine; LSD-25, psilocin and psilocybin; 3-hydroxytyramine; noradrenaline and adrenaline; BOL-148. The latter compound had a very weak action. Several experiments were carried out to test whether BOL-148 interfered with the effect produced by 5-HT and no antagonism could be detected. In addition to being less effective than 5-HT, some of the other active compounds were more prolonged in their action after the applying current was terminated. Thus the recovery from an application of LSD-25 using an electrophoretic current which produced depression in 30–40 sec. often took 10–15 min.

Several conclusions can be drawn from these observations.

The failure of 5-HT to affect either the antidromic firing of these neurones or the firing evoked by L-glutamic acid indicates that 5-HT does not significantly modify the membrane conductance of the cells. Thus it is very unlikely that it was combining with the sub-synaptic receptors of inhibitory synapses and activating the associated receptor membrane, or that it was depressing the electrically excitable membrane of the cells in some other fashion. It is probable that the depression of the orthodromic responses was due to a reversible interaction between 5-HT and the receptors at excitatory synapses which are specialized for the transmitter released from the optic tract terminals. However, the possibility that 5-HT was depressing the release of this transmitter cannot be excluded until the transmitter is identified and applied to the synapses.

The antagonism which is exhibited between 5-HT and the other compounds, when tested on smooth muscle, was not apparent at these central synapses. Indeed these 'antimetabolites'[3] had actions similar to that of 5-HT. Presumably all these depressants interacted with the same receptors, the potency and the duration of action of a particular compound

being determined by the stability of the drug-receptor complex.

The possible interaction of these substances with post-synaptic transmitter receptors suggests that the excitatory transmitter acting on the lateral geniculate neurones might be related structurally to them. Experiments are proceeding with a large number of indoles and catecholamines in order to determine those structural features necessary for a compound to interact with the transmitter receptors.

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Hormones and Electrolyte Excretion in Sheep

Merino sheep in hot environments effectively reduce the rate of excretion of water but that of sodium and potassium may not change, and often increases during periods of dehydration[1]. Renal hæmodynamics may be concerned in this adjustment of kidney function but endocrine activity has been examined in the search for possible mechanisms.

Merino ewes were accustomed to life in metabolic cages on a fixed feeding regime at room temperatures between 22° and 24° C. Electrolyte excretion was followed throughout periods of 36 hr. using a fraction collector to take specimens at ½ hr. intervals. There was considerable variation in excretion, mainly following morning feeding which produced a rise in sodium and potassium output by day and a low rate of excretion at night. Sheep were therefore trained to take lucerne chaff during a period of 3 hr. between 10 a.m. and 1 p.m. It was observed that within 20–30 min. of beginning to feed, normally alkaline (*p*H 8·2–8·6) urine became acid (*p*H 5·2–6·6) for about 1 hr. While feeding continued (Fig. 1*c*) the urine returned to a *p*H of 8·2. Production of saliva with a high bicarbonate content could leave the blood depleted of alkali and an acid urine could result.

Infusion of small amounts of hormone intravenously over an hour was undertaken. Six hormones were examined. Mixed arginine and lysine vasopressin ('Pitressin'), infused at the rate of 12 milliunits/min., had two types of effect. At urine flows lower than 1 ml./min. there was an increase of water, sodium, potassium and chloride excretion per unit of time (Fig. 1*a*). At flows greater than 2 ml./min. there was a reduction of flow of urine and increased output of electrolyte. The osmolar excretion-rate increased at both high and low levels of urine flow under the influence of vasopressin. This occurred both in the field (the tropics at 21° S. latitude) and in the laboratory. During diuresis the osmolarity of urine was 100–200 m.osmol./l., and at low flows it was 1,400–1,800 m.osmol. Vasopressin produced urine between 500 and 1,700 m.osmol./l. always more concentrated than plasma.

Lysine vasopressin gave identical results. Although vasopressin does not produce the changes of urine flow found in a hot environment, it could contribute to the sodium excretion which can occur in such circumstances. At both high and low rates of urine flow, vasopressin increased glomerular filtration (measured by creatinine clearance) so that differences in renal dynamics do not account for the phenomena. It appears that the effect of the polypeptide is on electrolyte movements (mainly potassium) in the tubule.

Infusion of adrenaline at a rate of 15 μgm./min. into sheep with urine flows of 0·4–1 ml./min. (Fig. 1*b*) led to excretion of 3–6 ml./min. of water with no increase of electrolyte output. In partially dehydrated animals there was little effect on water flow. Noradrenaline infused at a rate of 5 μgm./min. increased water, sodium and potassium excretion. The effect of adrenaline in increasing water excretion is the opposite of that found during dehydration. Aldosterone increased the re-absorption of sodium in sheep given salt supplements (Fig. 1*c*) but it did not increase potassium excretion as in carnivores or man[2]. There was no change in *p*H and little in water output under the influence of an infusion of 10–300 μgm. *d,l*-aldosterone in the course of an hour.

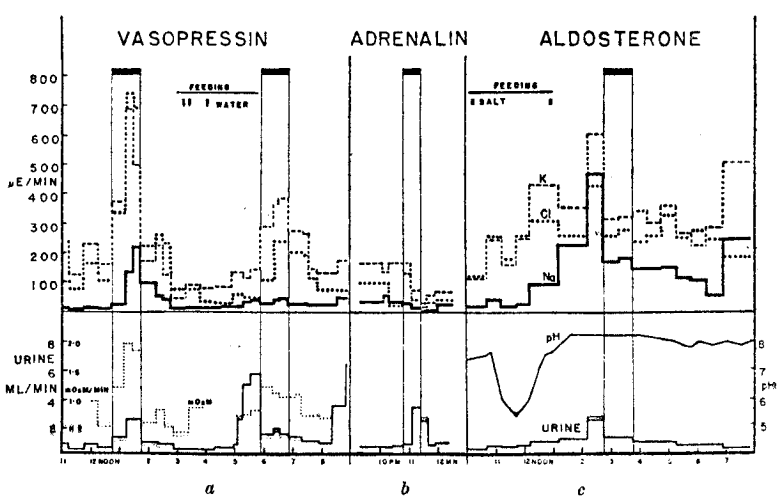

Fig. 1. Infusion of hormones intravenously in sheep resulted in urine excretory changes, those for potassium, chlorine and sodium being shown in the upper panel while water and osmolar excretion are shown below. *a*, Lysine vasopressin (12 milliunits/min.) increased low urine flow and decreased high urine flow, but osmolar and electrolyte flow increased in both. *b*, Adrenalin (15 μgm./min.) caused water to be excreted, but electrolytes were lost at a slower rate. *c*, During feeding, urine *p*H fell, but rapidly returned to the normal 8·2–8·5. Ingestion of sodium chloride in 2-gm. aliquots increased sodium excretion and this was reversed for 4 hr. by 0·5 μgm./min. *d,l*-aldosterone. Excretion of potassium was not increased by this substance