Effect of reserpine on cationic contents of rat brain

PREPARATIONS of the plant *Rauwolfia serpentina* have been used in India for centuries as a calming drug. Reserpine is the principal alkaloid of this plant and its effect on the central nervous system (CNS) has been studied extensively. The most important biochemical effect of reserpine on the CNS found *in vivo* is the release of noradrenaline, 5-hydroxytryptamine and dopamine from the amine storage sites in brain so that they are more susceptible to degradation by monoamine oxidase[1]. In synaptosomal preparations, reserpine has been shown to inhibit the uptake of noradrenaline and 5-hydroxytryptamine[2]. Prolonged reserpine treatment depletes these amines from the brain[1]. Amine depletion is thought to be due to changes in the permeability of neuronal membranes[3].

Tetrodotoxin and local anaesthetics increase the glycolysis of cerebral cortex slices[4] and this prompted us to investigate a number of neurotropic agents including reserpine. Studies with reserpine revealed that this drug can cause an increase in the potassium content of cerebral tissue both *in vitro* and *in vivo*. In view of the important role played by cations in the regulation of brain metabolism[5], the effect of reserpine on the potassium contents is of great interest and we are surprised that such measurements have not been made previously.

Rat cerebral cortex slices were prepared and incubated as described earlier[4,5] and anaerobic glycolysis, cationic contents, ^{22}Na transport and respiration were measured. For the *in vivo* experiments rats were given reserpine (2 mg kg^{-1}, Ciba) or saline intraperitoneally for 5 d after which they were killed, the brains quickly removed, weighed and protein-free extracts prepared for cation analysis by a Coleman flame photometer.

The *in vitro* experiments, details of which will be published elsewhere, showed that during anaerobic conditions, 12–120 µM reserpine greatly enhances the rate of glycolysis by adult rat and guinea pig cerebral cortex slices. The maximum effects observed were up to 3–4 times the normal rate. It was thought possible that reserpine may exert this effect indirectly by amine release. Experiments carried out with biogenic amines showed that unlike reserpine, these amines have no significant effect on anaerobic glycolysis. It was therefore concluded that reserpine may also exert its effect on the anaerobic glycolysis by increasing K^+/Na^+ ratio, as is the case with tetrodotoxin and local anaesthetics. This was found to be true and reserpine had a significant effect in increasing the K^+ contents of the cerebral cortex slices incubated under anoxic conditions, the increase being up to 34 µeq per g initial wet weight of the tissue as compared to 17 µeq per g initial wet weight for the controls.

Other experiments further demonstrated that 12 µM reserpine may affect the cationic movements. It was found that the respiration of rat cerebral cortex slices, stimulated by 10 µM protoveratrine, was suppressed by 12 µM reserpine. These effects were much more evident in a Ca^{2+}-free Krebs–Ringer phosphate medium. ^{22}Na movement was also suppressed by reserpine in these conditions.

A most interesting effect of reserpine was observed *in vivo* (Table 1). It was found that chronic reserpine administrations results in an increase in the K^+ contents of brain of more than 25% while the Na^+ contents are not affected. The cationic contents described here were determined in the whole brain. It is possible that most of the K^+ increase occurs intracellularly (and primarily in the neurones as compared to the glia), since very little extracellular space is present in brain.

The effect of reserpine on the amine uptake presumably results from changes in the permeability of neuronal membrane with a concomitant increase in the steady state level of K^+ Our results have wide implications. Sedation induced by reserpine is thought to result from a decrease in the quantity of monoamines[1]. High cerebral potassium in reserpinised animals may cause neuronal hyperpolarisation resulting in the potentiation of sedative effects. It has been argued that barbiturates and other sedatives may primarily act by inhibiting impulse conduction rather than amine metabolism[7].

Our observations also raise the question whether an increase in the level of cerebral K^+ is the primary effect of reserpine or is caused indirectly due to other central effects. The former possibility seems to be true. The uptake of amines by nerve endings is dependent on the presence of Na^+ (ref. 8), and the mechanism is activated by excitation and thus depolarisation[10]. Membrane affinity for neurotransmitters is affected by nervous stimulation[9]. Moreover, K^+ has been shown to compete with Na^+ for the Na^+-dependent uptake of monoamines[10,11]. Almost 80% of noradrenaline released at the synapse is inactivated by the uptake mechanism[9]. It seems that the increase in neuronal K^+ affects the uptake of amine by storage granules, presumably by competing with Na^+ (refs 10 and 11). Modulation of membrane affinity to neurotransmitters, therefore, seems to be involved in the action of reserpine. Further investigations are in progress to examine these possibilities.

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Table 1 Effect of chronic reserpine administration on the Na^+ and K^+ contents of rat brain

Treatment	K^+ (µeq per g fresh weight)	Na^+ (µeq per g fresh weight)
Control (12)	122.7 ± 5.7	48.5 ± 3.0
Experimental (12) (Reserpine, 2 mg kg^{-1} d^{-1} for 5 d)	157* ± 4.7	49.3† ± 2.5

K^+ and Na^+ contents were determined as given in the text. Values obtained, and their standard deviations are given. Number of observations are shown in parentheses.
*$P < 0.001$, highly significant.
†$P > 0.4$, not significant.

S-100 protein in synapses of the central nervous system

THE brain-specific S-100 protein[1] is predominantly localised in glial cells in the central nervous system and is synthesised by glial cells in tissue culture[2-5]. S-100 is, however, also present in neurones[6-9], located in both the neuronal perikaryon and the nucleus[6,9-11]. It has been proposed that the protein is transported along the axon[10] although the presence of S-100 in the Schwann cell[12,13] calls for confirmation of an intra-axonal localisation of the protein. Approxi-