Phenytoin, at a concentration which completely inhibits synaptosome Na^+,K^+-ATPase (5×10^{-5} M), decreased the basal release of phosphate. However, phenytoin did not alter basal acetylcholine release. When electrical pulses were applied acetylcholine release was increased considerably but phosphate release was also increased. Phenytoin prevented the evoked release of acetylcholine and it markedly reduced the evoked release of phosphate.

The question arises as to how much phosphate release is associated with Na^+,K^+-ATPase activity. Experiments involving a number of enzyme inhibitors suggested that approximately 25% of the basal release was limited to Na^+,K^+-ATPase while at least a further 65% resulted from the activities of other ATPases in the synaptosomes.

These results will be discussed in relation to the transmitter release-Na^+,K^+-ATPase hypothesis.

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Effect of reserpine on choline acetyltransferase and high affinity choline uptake in the rat brain

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Regulation of the synthesis of acetylcholine (ACh) in response to nerve impulse flow has been proposed in central (Grewaal & Quastel, 1973) and peripheral (Collier & MacIntosh, 1969) tissues, and adaptational changes affecting neuronal ACh synthesis have been described. Thus choline acetyltransferase (ChA) activities are increased during neuronal activation by drugs (e.g. Mandell & Knapp, 1971; Oesch, 1974). Similarly, changes have been reported in the maximum rate of sodium-dependent high affinity choline uptake (ChU) into brain synaptosomes (Simon, Atweh & Kuhar, 1976). No information was available, however, about the relationship between adaptations of this kind. Reserpine had been shown to decrease ACh concentrations (Beani *et al.*, 1966) and to increase ChA activities in brain (Mandell & Knapp, 1971). We therefore sought adaptations in ChA in

Table 1 Increase of striatal choline acetyltransferase (ChA) and choline uptake (ChU) after treatment of rats with reserpine

Treatment	*ChA activity (nmol/min, 10 mg protein) (1)*	*% of control activity*	*Sodium-dependent uptake of [³H]-choline (pmol/5 min, 10 mg tissue) (2)*	*% of control uptake*
Controls	10.1 ± 0.29	100	3.74 ± 0.076	100
Reserpine 14 h	10.1 ± 0.31	100	4.09 ± 0.18*	109*
18 h	11.9 ± 0.49	118*	4.51 ± 0.11‡	121‡
20 h	12.8 ± 0.86†	127†	4.79 ± 0.40‡	128‡
24 h	10.6 ± 0.41	105	3.73 ± 0.41	100

All ChU values were corrected for sodium-independent (low affinity) uptake by subtracting blanks measured after incubations in a sodium-free medium. [³H]-choline: 0.25 μM throughout.
(1) Mean of duplicate determinations on 6 rats ± s.e. mean; control group, mean of 24 rats.
(2) Mean of 4 determinations on 12 rats ± s.e. mean; control, 24 rats.
Student's 2-tail *t*-tests: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.002$.

response to reserpine, and compared the time course of adaptations in both ChU and ChA.

Male Wistar rats (150–200 g) were injected with reserpine (7.5 mg/kg i.p.). The dose was repeated after 12 h and the rats were killed 14 to 24 h after the first injection. ChA in brain homogenates (10% w/v, 50 mM-sodium phosphate, pH 7.0, 0°C) was assayed radiometrically (Fonnum, 1975). The uptake of [^{3}H]-choline into prism shaped ($0.1 \times 0.1 \times$ approximately 0.5 mm) tissue slices, incubated in Krebs medium (5 min, 37°C) was measured.

Homogenates of corpus striatum from animals treated with reserpine for 20 h showed statistically significant increases in ChA compared with controls (10.1 ± 0.48 to 12.8 ± 0.86 nmol/min, 10 mg protein, $P < 0.01$, $n = 12$). Activities in the cortex, midbrain, hypothalamus, cerebellum, and pons-medulla, however, did not show significant changes ($P > 0.05$). Striatal ChU was increased after 14 h and ChA after 18 h treatment. Increased values were found after 20 h and control values were re-established after 24 h treatment (Table 1).

Thus, the initial adaptation of striatal cholinergic neurones to reserpine involves an increase in ChU. There is a second phase, however, in which the increase in ChU is paralleled by an increase in ChA. This is consistent with the hypothesis that high affinity uptake and subsequent acetylation of choline are coupled in the rat (Barker & Mittag, 1975).

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The effects of chloroquine and other retinotoxic drugs on axonal transport of proteins in rabbit vagus nerve

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Human and animal studies have demonstrated ocular damage as a result of administration of chloroquine and hydroxychloroquine (Nylander, 1967; Shearer & Dubois, 1967), thioridazine (Zinn, 1975), ethambutol (Roberts, 1974) and clioquinol (see Meade, 1975). A number of these drugs, notably thioridazine bear a chemical similarity to chlorpromazine. Since chlorpromazine inhibits the axonal transport of proteins in peripheral nerves through an action on microtubules (Edström, Hansson & Norström, 1973; Cann & Hinman, 1974) we have examined the ability of the above retinotoxic drugs to inhibit the axonal transport of labelled proteins in rabbit vagus nerves *in vitro*.

Vagus nerves with nodose ganglia attached were incubated in medium 199 for 24 h at 38.5°C in a series of two-compartment chambers (McLean, Frizell & Sjöstrand, 1975). Fifteen μCi of tritium-labelled leucine (L-(4,5-3H) leucine, 58 Ci/mmol) were added to the compartments containing the nodose ganglia and a ligature tied on the nerves 6 cm from the ganglia at the start of the experiment. Drugs are dissolved in medium 199 and added to either nerve or ganglion compartment. Tritium-labelled proteins, transported from the nerve cell bodies in the ganglia accumulated in the axons proximal to the ligature. The amount of TCA-insoluble radioactivity in 5 mm segments of each nerve was measured by liquid scintillation counting and expressed as a fraction of the TCA-insoluble radioactivity in the rest of the nerve. Ganglia were homogenized and the TCA-insoluble radioactivity expressed in relation to protein content.

A significant ($P < 0.05$) decrease in the accumulation of proteins in the 5 mm segment of nerve immediately proximal to the ligature was found after treatment of the nerve trunks with chloroquine and hydroxychloroquine at a concentration of 10^{-3} M and with thioridazine, clioquinol and chlorpromazine