the mast-cell granule represent obvious parallels in other amine-storage granules.

Squid brain synaptosomes have been isolated (Dowdall & Whittaker, 1971) and found to contain about 60 times the concentration of acetylcholine in mammalian cortical synaptosome preparations. When incubated in sea-water with [^3H]choline (M. J. Dowdall & E. J. Simon, unpublished work) they take up the latter at about 100 times the rate of mammalian cortical synaptosomes by an Na^+ -dependent, cyanide-, dinitrophenol-, ouabain- and hemicholinium-sensitive carried-mediated uptake mechanism. Li^+ or K^+ cannot replace Na^+ . Kinetic analysis shows that two systems are apparently involved in choline uptake, a high-affinity ($K_m = 2-4 \mu\text{M}$) and a low affinity ($K_m > 24 \mu\text{M}$) system. It is thought that the high-affinity system may be that involved in the uptake of choline by cholinergic synaptosomes. Careful re-examination of published data for the uptake system in mammalian nervous tissue (Diamond & Kennedy, 1969; Adamič, 1970) suggests that here, too, two carriers may be involved, but the high-affinity system is largely masked by the low-affinity system, probably because of the low concentration of cholinergic terminals in these preparations. The rate of choline uptake declines in synaptosomes that have been stored for a few hours. Such synaptosomes show elevated cytoplasmic acetylcholine concentrations, and this suggests that choline uptake may be inhibited by acetylcholine in concentrations only a little higher than those normally found in the cytoplasm. If so, this may provide a clue to the regulation of acetylcholine synthesis in the terminal region. Externally applied acetylcholine is known to compete with choline for the choline carrier in mammalian synaptosomes (Marchbanks, 1969) and brain slices (E. Heilbronn, personal communication).

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Regulatory Significance of the Release and Action of Adenine Derivatives in Cerebral Systems

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Adenosine addition and electrical excitation are among the most effective means of augmenting the cyclic AMP (adenosine 3':5'-cyclic monophosphate) of tissues from the brain (Kakiuchi *et al.*, 1969; Sattin & Rall, 1970). The two are linked: electrical stimulation, among its other actions, releases adenosine from the tissues to extracellular fluids (McIlwain, 1971; Pull & McIlwain, 1972a,b). The present account takes for granted the involvement of cyclic AMP in metabolic regulation and queries: to what extent and in what manner may translocation of adenine derivatives play a part in conditioning the amounts and the actions of cyclic AMP, or in other aspects of metabolic regulation in the brain?

Accompanying the adenosine that appeared on excitation, and could be collected by superfusion, were adenine nucleotides, inosine and hypoxanthine (Pull & McIlwain, 1972a). Indeed, the nucleotides may be the form released, for rapid addition of AMP or of ATP during superfusion of neocortical tissues gave considerable conversion into adenosine, with some inosine and hypoxanthine though not adenine. Output of adenosine from the native or [^{14}C]adenine-labelled nucleotides increased with the frequency and duration of stimulating pulses; up to 60 nmol/g was collected as adenosine and some 160 nmol/g as adenosine plus inosine and hypoxanthine after 10 min excitation of guinea-pig cortical samples (Pull & McIlwain, 1972a, and unpublished work).

After the uptake of [^{14}C]adenine by incubated cerebral tissue, most ^{14}C is present in ATP (Santos *et al.*, 1968). The effluent adenosine and its metabolites from superfusion of such tissues were of low specific radioactivity, and increased several-fold on electrical excitation (I. Pull & H. McIlwain, unpublished work). Heterogeneity in pools of adenine derivatives in cerebral tissues has been demonstrated also by Sattin & Rall (1970) and Shimizu & Daly (1970). In particular, in such tissues, cyclic AMP was

formed from pools of high-specific-radioactivity ATP under the influence of noradrenaline or histamine. This suggested the ATP of high specific radioactivity to be at the postsynaptic sites associated with tissue responses to the neurohumoural agents, e.g. at neuronal cell bodies or large dendrites; some activity has been found in synaptosomal fractions (Y. Kuroda & H. McIlwain, unpublished work). Output of [^{14}C]adenosine from [^{14}C]adenine-loaded tissues was correlated with tissue responses to excitation induced electrically: it was diminished by $1\text{ }\mu\text{M}$ -tetrodotoxin and conditioned by the Ca^{2+} concentration of superfusing fluids (McIlwain & Pull, 1972; Pull & McIlwain, 1972a).

Adenosine potentiated the actions of noradrenaline or histamine in increasing the cyclic AMP of cerebral tissues: the increases were more than additive, as also were those resulting from joint action of electrical excitation and either of the two amines (Kakiuchi *et al.*, 1969; Sattin & Rall, 1970). Relationships among noradrenaline, histamine and serotonin (5-hydroxytryptamine) by contrast were additive; this indicated an independent and more general action on the part of adenosine. To some extent this was through adenosine acting as precursor, and not only catalyst, in the formation of cyclic AMP. Tissues incubated first with [^{14}C]adenine and then treated briefly with [^3H]adenosine yielded cyclic AMP containing both ^3H and ^{14}C (Shimizu & Daly, 1970).

Stimulation augments the cyclic AMP of sympathetic ganglia in postsynaptic regions, and this may be a feature applicable to other neural systems and neurohumoural agents (McAfee *et al.*, 1971; McIlwain, 1971). The more than additive actions of adenosine and noradrenaline are presumably due to joint impingement in some fashion on a common region. Thus adenosine, added or liberated in the tissue, can be pictured to increase the concentration of ATP available to a noradrenaline-activated adenylate cyclase; relevant K_m and concentration values have been appraised (McIlwain, 1971). The amount of adenosine liberated (see above) may correspond to 0.1 mM or more adenosine in limited extracellular spaces, and this concentration causes a rapid 20-fold increase in the cyclic AMP of cerebral tissues (Sattin & Rall, 1970).

It is thus noteworthy that liberation of ATP accompanies liberation of catecholamines in several neural systems (Douglas & Poisner, 1966; Geffen & Livett, 1971) and also that ATP or adenosine are postulated as neurotransmitters with action especially on smooth muscle (Holton, 1959; Burnstock *et al.*, 1970). The liberation of cyclic AMP on electrical excitation of cerebral tissues, though small, may have its independent significance, for an action on cell-firing has been observed (Siggins *et al.*, 1969). There are suggestions of further regulatory action

of adenosine through studies of regional blood flow (Haddy & Scott, 1968); in the brain this is increased by papaverine (McHenry *et al.*, 1970), which at $50\text{ }\mu\text{M}$ increases the adenosine appearing in superfusion fluids from stimulated tissues (Pull & McIlwain, 1972b).

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Adenosine 3':5'-Cyclic Monophosphate as a Mediator in the Action of Neurohumoral Agents

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Although cyclic AMP (adenosine 3':5'-cyclic monophosphate) has been shown to mediate a wide variety of hormonal effects in many different tissues, little information has been available for most tissues as to its mechanism of action. For that reason, studies were carried out to determine its chemical reactivity. Measurement of the equilibrium constant of the adenylate cyclase reaction:

led to the conclusion that the 3'-bond of cyclic AMP is of high energy content, with a free energy of