Clonidine as an adenosine antagonist

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It appears likely that several adenine derivatives including adenosine may be important in the normal functioning of the nervous system, either being released as neurotransmitters, or, more likely, released in a more non-specific way from excitable tissues during depolarization (McIlwain, 1972; Stone, 1978). However released, these purines can profoundly affect neural function by direct depression of firing rate or inhibition of neurotransmitter release (Stone, 1978).

Further knowledge of the role of these adenine compounds will require the availability of specific antagonists. It is known that several 2-substituted imidazoline derivatives such as phentolamine, antazoline, tolazoline and yohimbine will block peripheral effects of adenine derivatives (Satchell, Burnstock & Dann, 1973; Tomita & Watanabe, 1973) but these compounds are not specific and are therefore of limited use. Clonidine [2-(2,6-dichlorophenylamino)-2-imidazoline] is also a 2-substituted imidazoline compound which is known to act on the central nervous system (Schmitt, 1976), and we have therefore examined the effects of clonidine on neuronal responses to adenosine *in vivo*.

All compounds were applied by microiontophoresis to neurons in the motorsensory areas of the cerebral cortex, in rats anaesthetized with urethane, 1·25 g kg^{-1}. Drugs were applied from 7-barrelled micropipettes, from solutions of the following concentration: adenosine hemisulphate, 200 mM (pH 4·5); (−)-noradrenaline bitartrate, 200mM (pH 3·5); 5-hydroxytryptamine creatine sulphate (5-HT), 25mM (pH 4·5); clonidine HCl, 50mM (pH 4·0). The drugs were applied in a constant time cycle from a Digitimer Neurophore unit, which was also used to trigger the generation of post-stimulus time histograms by an Ortec Time Histogram Analyser. Action potentials were recorded by a separate single microelectrode glued alongside the multibarrel pipette (Stone, 1973).

All 26 neurons tested were depressed by adenosine applied with iontophoretic currents of 20 to 100 nA for 2 to 10 s (Phillis, Kostopoulos & Limacher, 1974; Stone & Taylor, 1977). At least one of the two control agonists used, noradrenaline and 5-HT, also depressed each cell tested.

When clonidine was ejected with currents greater than 25 nA, there was usually a marked depression of neuronal firing. This has been reported previously, in a study in which direct responses to clonidine were being sought (Anderson & Stone, 1974). However, the ejection of clonidine with currents of less than 25 nA resulted in a block of neuronal responses to adenosine on 22 of the 26 cells examined, and without any apparent change of the control amine responses. An example is illustrated in Fig. 1.

Two aspects of the blockade of adenosine by clonidine are particularly notable. Firstly, it was apparent at very low doses of clonidine. Simply removing the 15 nA retaining current which was normally applied between ejections to reduce spontaneous efflux of clonidine by diffusion was sufficient to block adenosine responses within 2 min on 6 cells. Secondly, when clonidine was applied with currents of 10–20 nA the blockade was rapid in onset, usually within 30 s, and equally quickly reversible when the clonidine ejection was terminated.

The present results suggest that clonidine blocks neuronal depressant responses to adenosine at doses which neither produce direct depression of cell firing, nor interference with noradrenaline of 5-HT. Clonidine may therefore prove useful in studying the functional role of adenines in the nervous system.

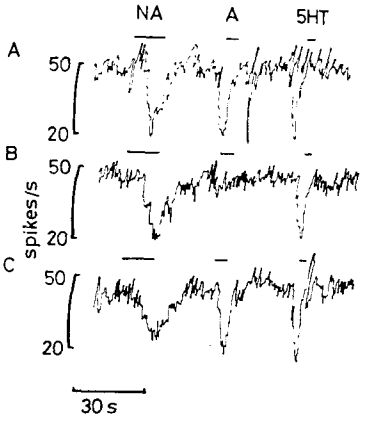

FIG. 1. Post-stimulus time histograms consisting of four summated sweeps, showing the firing rate of a neuron in response to the iontophoresis of noradrenaline, 60nA, (NA); adenosine, 50 nA, (A); and 5-hydroxytryptamine, 40 nA, (5-HT). The time of application of compounds is indicated by the bars above the records. The histograms were generated with a bin width of 0·5 s (total sweep duration 128 s). A, B and C: histograms generated respectively before, during, and after the application of clonidine, 15 nA. The first of the four sweeps comprising trace B was begun 1 min after starting the ejection of clonidine. Ejection was then maintained throughout the subsequent 3 sweeps. The first sweep of C was begun 1 min after ending the clonidine ejection.

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The relevance of this blockade of adenosine to the normal pharmacology of clonidine will require further work. However, although the drug is normally considered to be a central α-adrenoceptor agonist (Anderson & Stone, 1974; Schmitt, 1976) there are results not entirely compatible with such an action. Thus, clonidine is no longer effective after destroying central noradrenergic neurons (Dollery & Reid, 1973), its effects are not reduced by several α-blocking drugs (Schmitt, 1976) and it does not share with α-agonists the ability to activate cerebral adenylate cyclase (Skolnick & Daly, 1976).

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On the formation of 6-hydroxyindoles in rat brain

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As a major route of metabolism, tryptamine is oxidatively deaminated to 3-indoleacetic acid and, in addition, it has been shown that *in vitro* rat and rabbit liver microsomes can hydroxylate tryptamine to form 6-hydroxytryptamine (Szara & Axelrod, 1959; Jepson, Zaltzman & Udenfriend, 1962). More recently, Lemberger, Axelrod & Kopin (1971) have demonstrated the *in vivo* formation of 6-hydroxytryptamine in rabbit kidney and ileum as well as its occurence in urine. *N*-Methylated tryptamine has been demonstrated in rat brain *in vivo* and *in vitro* (Saavedra & Axelrod, 1972; Boulton & Baker, 1974).

The formation of 6-hydroxytryptamine in brain has not been demonstrated and, therefore, we considered it of interest to investigate the metabolic fate of tryptamine in rat brain with particular reference to oxidative hydroxylation as a metabolic pathway for cerebral tryptamine.

We now report that 6-hydroxytryptamine as well as 6-hydroxy-*N*-methyltryptamine can be formed from tryptamine in rat brain homogenates as well as 6-hydroxyindoleacetic acid and 3-indole-acetic acid which can be formed both *in vivo* and *in vitro*.

For the *in vitro* studies Albino rats (Wistar), 200–250 g were decapitated and the brains rapidly removed. After separation of the cerebral hemispheres and the cerebellum the remainder of the brain was homogenized in ten vol 0·32 M sucrose at 4°. The subcellular fractionation was done according to Michaelson & Whittaker (1963). The homogenates were centrifuged at 1000*g* for 10 min and the supernatants were centrifuged again at 17 000 *g* for 20 min to yield crude mitochondrial pellets. The crude microsomal pellets were obtained by centrifuging the remaining supernatant at 100 000 *g* for 60 min. All centrifugations were in a Sorvall refrigerated centrifuge or Spinco ultra-centrifuge at 4°. Mitochondrial or microsomal preparations were suspended in 3 ml incubation medium containing: 0·5 M phosphate buffer (pH 6·8), 1 μmol ATP (Sigma), 100 μg of tryptamine (Sigma), 0·5 μmol nicotinamide (Sigma). The medium was gassed with 5% CO_2 in oxygen. The mixture was incubated in a metabolic shaking incubator for 1·3 h at 37°. After adjusting the pH to 5·8, the mixture was allowed to react with sulphatase (arylsulphatase in combination with β-glucuronidase, Sigma). The incubation was for 6 h at 37°. To the medium containing the microsomal fraction was added 1 vol 0·5 M borate buffer (pH 10) and the mixture was extracted with 2 vol of benzene to remove unreacted tryptamine. The aqueous layer was saturated with sodium chloride and extracted with a mixture of n-butanol and isoamylalcohol (9 : 1).

The medium containing the mitochondrial fraction was adjusted to pH 1 (adding a few drops of con-

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