Br. J. Pharmac. (1978), **64**, 369–374

ANTAGONISM BY CLONIDINE OF NEURONAL DEPRESSANT RESPONSES TO ADENOSINE, ADENOSINE-5'-MONOPHOSPHATE AND ADENOSINE TRIPHOSPHATE

TREVOR W. STONE & DAVID A. TAYLOR*

Department of Physiology, St. George's Hospital Medical School, University of London, London SW17 0RE and
*Department of Pharmacology, University of Colorado Medical Center, Denver, Co., U.S.A.

1 Adenosine and its nucleotides adenosine-5'-monophosphate (AMP) and adenosine triphosphate (ATP) have been applied by microiontophoresis to neurones in the cerebral cortex of rats anaesthetized with urethane. The firing rate of most neurones was depressed, though two cells were encountered which showed biphasic responses to ATP consisting of an initial excitation succeeded by depression.

2 The application of clonidine with iontophoretic currents of less than 25 nA resulted in blockade of the depressant responses to the purines, without affecting responses to noradrenaline, 5-hydroxytryptamine or γ-aminobutyric acid (GABA). At much higher doses of clonidine, direct depression of cell firing occurred and occasional interaction with noradrenaline was noted.

3 In the case of the biphasic responses to ATP, clonidine seemed to block only the depressant phase. Reduction of the excitatory component paralleled changes of background firing.

4 It is concluded that, in common with some other 2-substituted imidazoline derivatives, clonidine possesses the ability to block responses to purine compounds.

Introduction

There is growing evidence that certain adenine derivatives such as adenosine and the nucleotides 5'-adenosine monophosphate (AMP) and adenosine triphosphate (ATP) may have some functional role in the nervous system (McIlwain, 1972; 1976; Stone, 1978). These purines are not only ubiquitously present in neural tissue, but they are also released from neurones by depolarizing stimuli such as potassium ions, dicarboxylic amino acids and electrical stimulation (Pull & McIlwain, 1972; Shimizu & Daly, 1972). They have potent effects on neurones, causing depression of the firing rate of central neurones (Kostopoulos & Phillis, 1977; Stone & Taylor, 1977; 1978) and inhibiting transmitter release from cholinergic and adrenergic synapses in peripheral and central neurones (Ginsborg & Hirst, 1972; Vizi & Knoll, 1976; Hedqvist & Fredholm, 1976; Clanachan, Johns & Paton, 1977).

Among the compounds reported to antagonize the effects of adenosine and its derivatives are 2-substituted imidazolines such as phentolamine, tolazoline, antazoline and yohimbine (Satchell, Burnstock & Dann, 1973). As clonidine is also a 2-imidazoline derivative we have examined this drug for possible antagonistic properties towards adenosine on single neurones *in vivo*.

Methods

Male Porton Wistar rats weighing 250 to 350 g were anaesthetized with urethane (1.25 g./kg. i.p.). The rat was placed in a stereotaxic frame and the body temperature maintained at 37 to 38°C by means of an automatically regulated heating blanket and a rectal probe. An area approximately 4 mm square of cerebral cortex was exposed immediately anterior to the bregma suture, to allow access to the motorsensory cortex. The dura was removed and, after positioning the electrodes, the exposed cortex and muscles were covered with a layer of 5% agar in 0.9% w/v NaCl solution (saline) to prevent cooling and drying, and to reduce respiratory and vascular movements of the brain. The agar layer was changed after each micro-

electrode penetration, though usually only one or two penetrations were made per experiment.

For the microiontophoretic application of compounds, 7-barrelled micropipettes were used. The barrels were filled immediately before use to approximately 1 cm from the top of the pipette. The centre barrel was always left unfilled. The pipettes were broken under microscopic control so the overall tip diameter was 10 to 15 μm. Iontophoretic ejection was effected by a Digitimer Neurophore unit, incorporating automatic balancing at the electrode tip. Current flow to ground, and thus current artifact, was thereby minimized.

For iontophoresis, the following solutions were used: (−)-noradrenaline bitartrate, 0.5 M, pH 3.5; γ-aminobutyric acid, 0.2 M, pH 3.5; 5-hydroxytryptamine creatinine sulphate, 25 mM, pH 4.0; adenosine hemisulphate, 0.2 M, pH 4.5; adenosine 5′-monophosphoric acid sodium salt, 0.2 M, pH 5.0; adenosine triphosphate sodium salt, 0.2 M, pH adjusted to 7.0 with NaOH and clonidine hydrochloride, 50 mM, pH 4.0. When not being ejected, compounds were subjected to a retaining current of 10 to 15 nA.

Extracellular recordings of unit activity were made with single glass microelectrodes containing 1 M potassium acetate or chloride, having d.c. resistances of 2 to 8 MΩ. The tips of these electrodes were bent to an angle of 10 to 20° during the pulling process to facilitate fixing alongside the multibarrel pipette (Stone, 1973). The tips of the single and multibarrel pipettes were initially approximated by eye, and then under microscopic control. During this stage the electrodes were held together by Plasticine, and permanent fixing was then achieved by an epoxy-resin. Tip approximation was always confirmed microscopically before and after each experimental penetration. The recording electrode was arranged to project 10–25 μm beyond the multibarrel tip.

Unit activity was amplified by a Grass P511 amplifier, and the spikes were passed through a window discriminator, the output pulses of which were counted and displayed as a record of spikes per second on a Grass polygraph. Spikes were simultaneously displayed on oscilloscopes and were also available for recording on magnetic tape and for online generation of post-stimulus time histograms by an Ortec time histogram analyser.

Some of the neurones tested were identified as pyramidal tract cells either by antidromic invasion from the medullary pyramid (Stone, 1972b) or by virtue of their characteristic excitatory responses to iontophoretically applied acetylcholine (Stone, 1972a). However, there was no difference apparent between the responses of these cells and of other non-identified units. No distinction has therefore been made in describing the results. All the units studied were spontaneously active.

Results

Adenine derivatives

Adenosine was applied to 26 neurones, all of which were depressed at iontophoretic doses of 20 to 100 nA applied for 5 to 15 s. The adenosine responses were usually apparent within 5 s of beginning the iontophoretic ejection, and recovered to the control rate within 30 s of terminating the ejection.

Adenosine-5′-monophosphate (AMP) produced closely similar responses and at comparable iontophoretic doses, on 16 of 20 neurones (Figure 1). Adenosine triphosphate (ATP) was applied to 20 units, and produced pure depression of 15 of these when ejected with currents of 20 to 60 nA for 2 to 12 s (Figure 2). Two neurones showed a biphasic response to ATP, consisting of an initial excitation followed by depression (Figure 3). This will be discussed again below.

Clonidine

When applied with iontophoretic currents greater than about 25 nA, clonidine induced a depression of neuronal firing on 6 of 11 units. Although the latencies of these responses were between 1 and 16 s, the depressions were much slower in reaching a maximum than were the purine responses. However, at currents less than 25 nA clonidine could be applied for several minutes with no resultant effects on firing rate or spike height.

Effect of clonidine on adenine responses

At doses less then 25 nA, clonidine proved able to antagonize readily the depressant effects of adenosine or AMP. An example is illustrated in Figure 1, which also exemplifies two notable characteristics of this blockade, the first being the potency of the antagonistic action. In this example, no ejecting current was applied to the clonidine barrel, but the 15 nA retaining current normally applied between ejections to reduce spontaneous diffusional efflux of the drug was removed, thus allowing relatively free diffusion from the pipette tip. With this procedure, antagonism towards adenosine was apparent on 4 units.

The second characteristic of the clonidine antagonism was the rapid onset and reversibility of the blockade. In Figure 1, the AMP response obtained 15 s after starting the clonidine leak is profoundly reduced, the responses returning equally quickly after restoring the clonidine retaining current.

Reduction of adenosine responses by clonidine (0 to 25 nA) was observed on 21 of 25 neurones, and of AMP responses on 6 of 8 neurones.

A record of one of the cells depressed by ATP is

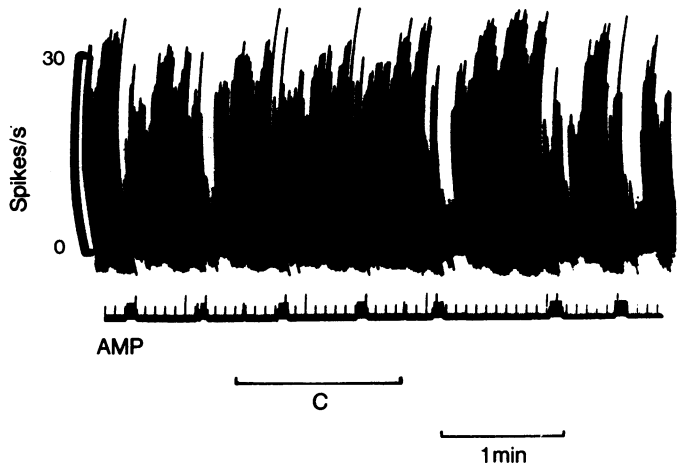

Figure 1 Record of the firing rate of a neurone in the cerebral cortex, depressed by iontophoretic applications of adenosine-5′-monophosphate (AMP) with a current of 40 nA. During the period indicated by the bar (marked C), the inward retaining current was removed from the clonidine containing barrel, allowing diffusion from the pipette tip. The AMP responses were markedly reduced in size, but recovered rapidly on restoring the clonidine retaining current.

Figure 2 Record of the firing rate of a cortical neurone showing depressant responses to adenosine triphosphate (ATP, 50 nA). The ejection of clonidine, 20 nA (C) substantially reduced these responses, which then began to recover soon after clonidine application. This cell was lost before complete recovery occurred, but is interesting in view of the appearance of a brief excitatory component of the ATP response, during blockade of the depressant phase.

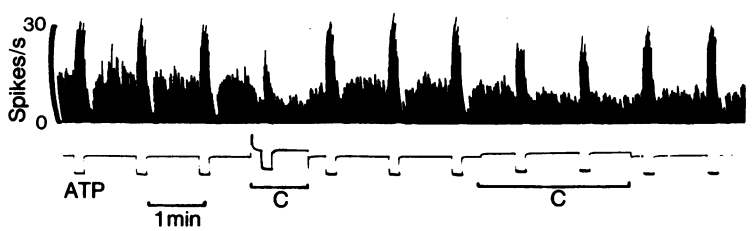

Figure 3 Record of the firing rate of a cortical neurone showing biphasic excitatory-depressant responses to adenosine triphosphate (ATP, 60 nA). The first application of clonidine (C) was made using a current of 40 nA. There is a marked depression of background firing and of the excitatory phase, though the relative increase of rate (approx. 110%) remained unchanged. Immediately after the clonidine ejection, at a time when the excitation and background discharge have reached control size, the depressant phase remains blocked. A similar sequence appears later in the record, when a smaller dose of 25 nA of clonidine was used.

shown in Figure 2, and the blockade of the response by 20 nA of clonidine is readily apparent. However, it should be noted that during the blockade of the depressions, there arises a brief excitatory effect of ATP, which disappears towards the end of the record, as the depressant response recovers. This neurone was lost before complete recovery occurred.

The firing rate of one of the two neurones exhibiting a biphasic response to ATP is shown in Figure 3. Two tests with clonidine are shown, in which currents of 40 nA and 25 nA respectively were used. There is a reduction in the size of the excitatory component during the clonidine application, which is probably a result of the reduction of background firing rate. An examination of the first ATP responses after each clonidine application indicates that the depressant phase is blocked although the firing rate and excitatory component have returned to normal.

Specificity of the blockade

At least one of the control depressant compounds noradrenaline, 5-hydroxytryptamine and GABA depressed each of the neurones studied. At the doses of less than 25 nA of clonidine used to reduce adenine responses, none of these control substances was affected on any of the cells (Figure 4). Some interaction with noradrenaline was occasionally seen when high doses of clonidine of 80 to 100 nA were applied for 2 or more min. This interaction sometimes took the form of an antagonism (2 of 18 cells) and sometimes of a potentiation (2 of 18 cells). Even at these high doses of clonidine, therefore, interactions with noradrenaline were infrequent. It should be pointed out that these doses of clonidine also usually resulted in a direct inhibition of the cells (Anderson & Stone, 1974) on occasions associated with reduction of spike height. No effect of clonidine was seen on responses to 5-hydroxytryptamine (16 cells) or GABA (12 cells).

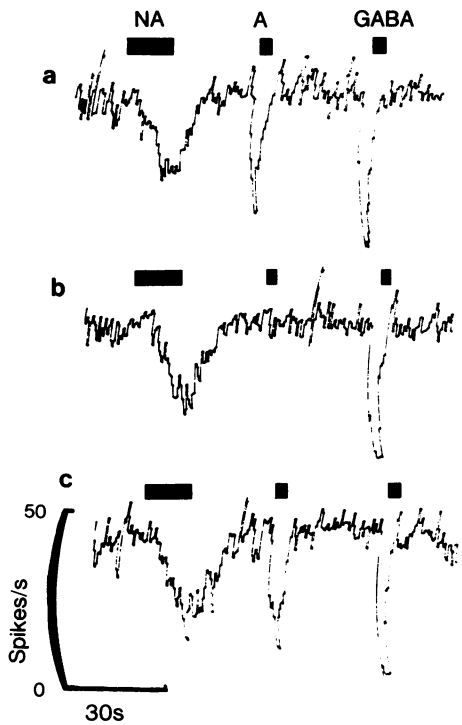

Figure 4 Post-stimulus time histograms of neuronal firing rate in response to the iontophoresis of noradrenaline, 60 nA (NA), adenosine, 50 nA (A) and γ-aminobutyric acid, 30 nA (GABA). Each trace consists of four summated sweeps triggered by the iontophoretic apparatus. Bin width is 0.5 s (total sweep duration 128 s). (a), (b) and (c): before, during and after the application of clonidine, 15 nA, for 10 min.

Discussion

The responses of neurones to the adenine derivatives used in this study are similar to those which have been described previously (Phillis, Kostopoulos & Limacher, 1974; Kostopoulos & Phillis, 1977; Stone & Taylor, 1977; 1978; Taylor & Stone, 1978). Depressant responses were rapid in onset and recovery, and occasional excitatory responses to ATP were observed (Phillis *et al.*, 1974).

A previous examination of the effects of clonidine alone on individual neurones led to the observation of primarily depressant responses at doses of 60 to 80 nA of the drug (Anderson & Stone, 1974). The depression of unit firing by doses of more than 25 nA in the present study stands in confirmation of this.

The main finding in the present paper is the marked sensitivity to antagonism by clonidine of depressant responses to purine compounds. Leaking clonidine by diffusion from the micropipette was sufficient to block purine responses within seconds, with rapid recovery. Only at much higher iontophoretic currents was any interference seen with noradrenaline depressions, and even then interaction was of variable direction and occurred on only 4 of 18 cells. 5-Hydroxytryptamine and GABA never seemed to be affected by clonidine, although, as we have noted, the direct depression of firing by the higher doses of clonidine, frequently complicated interpretation of the records.

We conclude that there is a substantial degree of selectivity in the blockade of purines by clonidine. This antagonism may result from the 2-substituted imidazoline structure of clonidine, a structure shared

with compounds such as phentolamine, tolazoline, antazoline and yohimbine which have been reported to block ATP responses in peripheral tissues (Satchell *et al.*, 1973). In unpublished experiments we have confirmed that phentolamine will block depressant responses of cortical neurones to adenosine, though at doses of 20 to 60 nA for 2 to 10 min: doses which often depress firing rate and spike height.

The blocking action of clonidine appears to be directed particularly at the depressant components of purine responses. Excitatory responses to ATP have been occasionally described in the cerebral cortex (Phillis *et al.*, 1974) and we have confirmed their occurrence, but any reduction by clonidine of the size of the excitatory phase seems to occur *pari passu* with depression of background discharge and we have no evidence from the two excited neurones recorded of any specific pharmacological blockade. In contrast, the depressant phases of biphasic responses remained blocked for a short time after the clonidine application, consistent with the idea of a specific antagonism. It remains to be determined whether this distinction is indicative of two kinds of receptor for ATP mediating the two components, only one of which (mediating depression) is susceptible to block by clonidine.

It might be expected that such an apparently potent action of clonidine would contribute to the centrally-mediated hypotensive properties of the drug for which it is used clinically. Many of the *in vivo* studies have produced results consistent with a mechanism involving an agonist action at central α-adrenoceptors, and this seems to be the most widely held view (Schmitt, 1976). However, there are many results not wholly compatible with such an explanation, including the failure of many α-antagonists to block clonidine's effects (Schmitt, 1976), the abolition of the hypotensive effect by 6-hydroxydopamine (Dollery & Reid, 1973), and the absence of an agonist action of clonidine on adenylate cyclase preparations (Skolnick & Daly, 1976). Indeed, in the latter paper, clonidine was found to act as an α-antagonist.

The present demonstration of an ability of clonidine to antagonize responses to adenine derivatives, increasingly thought to play an important role in the CNS (McIlwain, 1976; Stone, 1978) may indicate an alternative mechanism of clonidine action which may allow a resolution of some of these discrepancies. Clonidine in turn may be a useful tool in elucidating the physiological role of adenine derivatives in the nervous system.

We are grateful to Boehringer Ingelheim Ltd., for the gift of clonidine, and to the Royal Society and Wellcome Trust for travel grants.

References

ANDERSON, C. & STONE, T.W. (1974). On the mechanism of action of clonidine: effects of single central neurones. *Br. J. Pharmac.*, **51**, 359–365.

CLANACHAN, A.S., JOHNS, A. & PATON, D.M. (1977). Presynaptic inhibitory actions of adenine nucleotides and adenosine on neurotransmission in the rat vas deferens. *Neurosci.*, **2**, 597–602.

DOLLERY, C.T. & REID, J.L. (1973). Central noradrenergic neurones and the cardiovascular actions of clonidine in the rabbit. *Br. J. Pharmac.*, **47**, 206–216.

GINSBORG, B.L. & HIRST, G.D.S. (1972). The effect of adenosine on the release of the transmitter from the phrenic nerve of the rat. *J. Physiol.*, **224**, 629–645.

HEDQVIST, P. & FREDHOLM, B.B. (1976). Effects of adenosine on adrenergic neurotransmission: prejunctional inhibition and postjunctional enhancement. *Naunyn Schmiedebergs Arch. Pharmac.*, **293**, 217–224.

KOSTOPOULOS, G.K. & PHILLIS, J.W. (1977). Purinergic depression of neurones in different areas of the rat brain. *Expl Neurol.*, **55**, 719–724.

McILWAIN, H. (1972). Regulatory significance of release and action of adenine derivatives in cerebral systems. *Biochem. Soc. Sympos.*, **36**, 69–85.

McILWAIN, H. (1976). Translocation of neural modulators. A second category of nerve signal. *Neurochem. Res.*, **1**, 351–368.

PHILLIS, J.W., KOSTOPOULOS, G.K. & LIMACHER, J.J. (1974). Depression of corticospinal cells by various purines and pyrimidines. *Can. J. Physiol. Pharmac.*, **52**, 1226–1229.

PULL, I. & McILWAIN, H. (1972). Adenine derivatives as neurohumoral agents in the brain. The quantities liberated on excitation of superfused cerebral tissues. *Biochem. J.*, **130**, 975–981.

SATCHELL, D., BURNSTOCK, G. & DANN, P. (1973). Antagonism of the effects of purinergic nerve stimulation and exogenously applied ATP on the guinea-pig taenia coli by 2-substituted imidazolines and related compounds. *Eur. J. Pharmac.*, **23**, 264–269.

SCHMITT, H. (1976). Influence of adrenergic and cholinergic mechanisms on the central cardiovascular structures and their interactions. In *Drugs and Central Synaptic Transmission.* ed. Bradley, P.B. & Dhawan, B.N. pp. 63–88. London: Macmillan.

SHIMIZU, H. & DALY, J.W. (1972). Effect of depolarizing agents on accumulation of cyclic AMP in cerebral cortical slices. *Eur. J. Pharmac.*, **17**, 240–252.

SKOLNICK, P. & DALY, J.W. (1976). Interaction of clonidine with pre- and post-synaptic adrenergic receptors of rat brain: effects on cyclic AMP-generating systems. *Eur. J. Pharmac.*, **39**, 11–21.

STONE, T.W. (1972a) Cholinergic mechanisms in the rat somatosensory cerebral cortex. *J. Physiol.*, **225**, 485–499.

STONE, T.W. (1972b). Cotical responses to pyramidal tract stimulation in the rat. *Expl Neurol.*, **35,** 492–502.

STONE, T.W. (1973) Cortical pyramidal tract interneurones and their sensitivity to L-glutamic acid. *J. Physiol.*, **233,** 211–225.

STONE, T.W. (1978). Possible roles for purine compounds in neuronal adaptation. *Biochem. Soc. Trans.*, (in press).

STONE, T.W. & TAYLOR, D.A. (1977). Microiontophoretic studies of the effects of cyclic nucleotides on excitability of neurones in rat cerebral cortex. *J. Physiol.*, **266,** 523–543.

STONE, T.W. & TAYLOR, D.A. (1978). An electrophysiological demonstration of a synergistic interaction between norepinephrine and adenosine in the cerebral cortex. *Brain Res.*, **147,** 396–400.

TAYLOR, D.A. & STONE, T.W. (1978). Neuronal responses to extracellularly applied cyclic AMP: role of the adenosine receptor. *Experientia*, **34,** 481–482.

VIZI, E. & KNOLL, J. (1976). The inhibitory effect of adenosine and related nucleotides on the release of acetylcholine. *Neurosci.*, **1,** 391–398.

(*Received May 24, 1978.*)