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SHORT COMMUNICATIONS

The effect of a 5HT agonist on cyclic guanosine monophosphate in rat cerebellum

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The integrity of both the striatum [9] and cerebellum [10] is known to be required for maintenance of normal motor movement and control of posture. The function of the nigrostriatal system depends on intact dopaminergic nerve pathways. The high concentration of cyclic guanosine monophosphate (*c*GMP) in the cerebellum relative to other areas of the brain [6] and recent evidence that this nucleotide is implicated in cerebellar neuronal transmission [13] could indicate that *c*GMP has also a significant role in motor control. Despite the lack of dopamine (DA) input [16] to and the absence of DA receptors [5] in the cerebellum, there does seem to be some association of changes in activity of DA neurones in the basal ganglia with *c*GMP metabolism in the cerebellum. Apomorphine, for instance, a known DA agonist, increases *c*GMP in the cerebellum *in vivo* [3] and antipsychotics which block DA receptors and inhibit DAergic activity decrease cerebellar *c*GMP [1]. These data lead the authors to conclude that there is a neuronal pathway which mediates the dopamine control of cerebellar activity [1, 3].

The interaction between 5-hydroxytryptaminergic activity and DA mediated motor activity [7, 8, 17] indicates that changes in 5-hydroxytryptamine (5HT) metabolism may also be reflected in changes in cerebellar *c*GMP concentrations. In the present study, this possibility was investigated by determining the effect of 5-methoxy-dimethyltryptamine (5MeODMT), a suggested 5HT agonist [7], on the concentration of *c*GMP in rat cerebellum.

Female albino Porton Wistar rats, weighing 160–200g, were used throughout this study. They were injected intraperitoneally with 5MeODMT (10mg/kg) or physiological saline 10 or 30 min prior to killing. Cyproheptadine (10mg/kg) and methysergide (20mg/kg), both believed to act partly as 5HT receptor blockers, and haloperidol (1mg/kg), a dopamine receptor blocker, were administered i.p. for the appropriate experiment 30 min prior to 5MeODMT, and the control animals were injected with saline. For all experiments, animals were killed between 9:00 a.m. and 11:00 a.m., by cervical dislocation. Following decapitation, the cerebellum alone was quickly removed and dropped into liquid nitrogen. The tissue samples were homogenized in ice cold 6% TCA, and measured aliquots removed for protein assay [12] and for nucleotide extraction.

The aliquot of homogenate was centrifuged at 10,000*g* for 3 min and the supernatant removed for washing with water-saturated diethyl ether. The remaining ether was aspirated under a stream of nitrogen and the resultant aqueous phase buffered with Tris–EDTA, pH 7.5 (0.05M), before storing at −20° until analysis. *c*GMP was assayed in triplicate by the radioimmunoassay system from Radiochemical Centre, Amersham. The final data was analysed statistically using the Student's *t*-test.

The values for *c*GMP concentration in the cerebella of control animals (Table 1) are in agreement with the values obtained from mouse cerebellum [15]. It is clear that 5MeODMT significantly increases cerebellar *c*GMP by approximately 150 per cent and that this increase is maintained over at least 30 min. There is no effect of cyproheptadine, methysergide or haloperidol when administered prior to the injection of 5MeODMT.

The results obtained in this study indicate that a 5HT-like agonist can elicit an increase in *c*GMP in the cerebellum. This increase is of the same order as that found after apomorphine [3], but the lack of effect of haloperidol (1mg/kg) on the 5MeODMT-stimulated increase in *c*GMP indicates, however, the existence of a neuronal pathway which overrides dopaminergic activity. Microiontophoretic administration of 5HT has indicated the possible existence of a 5HT mediated neuronal pathway from the raphe to the cerebellum [2], and *c*GMP could be a second messenger in a 5HT neuronal pathway [14]. Thus the increase in

Table 1. *c*GMP concentration in rat cerebellum after 5MeODMT with or without DA or 5HT antagonists*

	*c*GMP (pmoles/mg protein ± S.D.)	
Control	10.8 ± 2.1	(4)
5MeODMT 10 min‡	25.7 ± 4.1	(4)
5MeODMT 30 min‡	26.1 ± 3.8	(5)
Haloperidol 30 min†, 5MeODMT 10 min‡	22.1 ± 3.2	(4)
Cyproheptadine 10 min†, 5MeODMT 10 min‡	25.1 ± 6.0	(4)
Methysergide 10 min†, 5MeODMT 10 min‡	26.4 ± 5.2	(5)
Methysergide 30 min†, 5MeODMT 10 min‡	25.8 ± 2.9	(4)

* The numbers in brackets indicate number of animals.
All values of *c*GMP were significantly different from control at $P < 0.001$. Dose levels: 5MeODMT, 10mg/kg; Haloperidol, 1mg/kg; Cyproheptadine, 10mg/kg; Methysergide, 20mg/kg.
† Time interval between first drug and second drug.
‡ Time interval between 5MeODMT and killing.

cGMP may be the result of a direct stimulation of the raphe-cerebellar pathway.

The capacity of the 5HT receptor blockers, cyproheptadine and methysergide, to block receptors in the cerebellum has not been investigated, but it has been shown that neither of the drugs are complete blockers of 5HT receptors in the CNS [2, 10]. Such a reduced efficiency may account for the lack of effect of these drugs in terms of blocking 5MeODMT-stimulated increase in cerebellar cGMP.

In conclusion, these data indicate that the concentration of cerebellar cGMP may be controlled by a 5HT mediated pathway which is not blocked by cyproheptadine or methysergide, and which does not appear to require the activation of an intermediate DAergic neurone.

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Department of Psychiatry, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, NE1 4LP, U.K.

ELEFTHERIOS LYKOURAS
DONALD ECCLESTON
ELIZABETH F. MARSHALL

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Stimulation of gluconeogenesis by adenosine in renal cortical tubule fragments from fed rats

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Adenosine has a diverse range of effects upon the physiological and biochemical function of many tissues (see review by Arch and Newsholme [1]). These range from 'hormone-like' effects seen at low concentrations to direct interventions in adenine nucleotide metabolism seen at relatively high concentrations (i.e. approximately 10^{-3}M). In the course of experiments designed to test whether adenosine might alter the response of renal gluconeogenesis to stimulating agents (Ca^{2+}, 3′, 5′ cyclic AMP or α-adrenergic agonists), it was observed that adenosine consistently increased the rate of the process above that seen in the basal state. This effect of adenosine, which was dose-dependent, was surprising and of interest since, by contrast, adenosine causes a considerable decrease in glucose formation from lactate in rat hepatocytes [2].

Chemicals were obtained as described by Macdonald and Saggerson [3]. In addition, adenosine was from Boehringer. Renal cortical tubule fragments were isolated by collagenase treatment of cortex pieces obtained from male Sprague–Dawley rats (160–180g body wt) as described previously [3] using Krebs–Ringer bicarbonate containing 1.27 mM Ca^{2+} as the incubation medium. Finally each preparation of tubules was made up in this medium to give a stock suspension in which the tissue from each original kidney cortex was dispersed in 5 ml. Aliquots (1 ml) of this were then taken for incubation at 37° as described by Macdonald and Saggerson [3] in a final volume of 4 ml with constant shaking under $O_2 + CO_2$ mixture (95.5%).

After incubation glucose was measured enzymically [4] in extracts prepared by deproteinization of incubation flask contents with 0.5 ml of ice-cold 45% (w/v) $HClO_4$. These extracts were neutralized by addition of 1M triethanolamine hydrochloride and concentrated K_2CO_3. In all experiments, the small amount of glucose present initially in non-incubated aliquots of tubules was also determined and subtracted from experimental values. The average basal rate of glucose formation was 2.7 μmole/hr/mg DNA. DNA was measured by the method of Burton [5] in 1.0 ml portions of stock tubule suspensions which generally contained 150–200 μg DNA.

Statistical significance was determined by Student's *t*-test on a paired basis.

Figure 1 shows that adenosine stimulated gluconeogenesis from lactate in a dose-dependent manner. Adenosine (10μm) was sufficient to achieve a significant acceleration of the process and in three out of four experiments 1 μM adenosine was also stimulatory. Larger effects were seen with 0.1 mM and 1 mM, the response with 1 mM being more variable. These observations were surprising in view of the finding of Lund *et al.* [2] that adenosine inhibits gluconeogenesis from lactate in rat hepatocytes. This would appear to suggest a clear and interesting difference between