even 6 weeks after pinealectomy also indicated the independent synthesis of NAS in this tissue[3]. Using PCPA treatment we have expected a decrease of NAI positive substance in the granule layer of cerebellum. The opposite findings seems to contradict the established effect of PCPA on serotonin synthesis. However this paradoxical observation is not unique. In 7 brain areas PCPA was reported to decrease the content of serotonin (range 31–46%). On the other hand in cerebellum the serotonin was increased by 44%[12]. AGHAJANIAN et al.[13] discovered that PCPA was not able to prevent the L-tryptophan induced increase of serotonin fluorescence of the neurons of the raphe system, even though the region of terminals in the forebrain was substantially depleted. In 1974 HARVEY and GAL[14] observed no blockade of TR-5-OHase in the septal region after PCPA. The authors of these two findings offer two different explanations of our unexpected results. AGHAJANIAN et al. hypothesized that synthesis of serotonin by PCPA is blocked in the nerve terminals while in the perikaryon of the neurons the serotonin production continues due to new synthesis of TR-5-OHase. The same explanation may hold for granule cells. Together with a feedback response to depletion of serotonin synthesis in the terminals of the granule cells it could even explain an increase of serotonin synthesis in the granule cells, supplying the substrate for increased NAS synthesis. Another possible explanation is the speculation of HARVEY and GAL[14] that at least 2 TR-5-OHase exist, one which is blocked by PCPA and the other which is not. This explanation does not provide any reason for increase in NAS.

The third possibility is an as yet unknown metabolic pathway for NAS synthesis which does not involve TR-5-OHase. Such a pathway might involve the metabolism of tryptophan to tryptamine, then to N-acetyltryptamine and finally hydroxylation to NAS by an unidentified reaction. However such a pathway seems less probable in view of recent findings of L. HSU et al. (personal communication). They observed an increase of N-acetyltransferase in the rat cerebellum after PCA treatment. Although this report correlated with our findings it does not help to explain the mechanism by which NAS is synthesized in the granule layer of cerebellum after PCPA. We hope that further investigations will contribute to the solution of this problem.

[12] E. D. WEITZMAN, M. RAPPORT, P. MCGREGOR and J. JACOBY, Science *160*, 1361 (1968).
[13] G. K. AGHAJANIAN, M. J. KUHAR and R. H. ROTH, Brain Res. *54*, 851 (1973).
[14] J. A. HARVEY and E. M. GAL, Science *183*, 869 (1974).

Is Glutamic Acid the Pyramidal Tract Neurotransmitter?

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Summary. Applied by microiontophoresis, 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) antagonized excitation by glutamic acid but not by acetylcholine of neurones in the rat cuneate nucleus. HA-966 blocked the short latency excitation of cuneate neurones following stimulation of the pyramidal tract on 28 of 40 cells (70%). Thus, glutamate or a related amino-acid may be the neurotransmitter released by pyramidal tract neurones.

The pyramidal tracts have at least two important physiological functions. They are involved in the control of fine movements of distal limb muscles[2], and they modulate the amount of sensory information reaching higher centres such as the cerebral cortex[3]. Yet little attention has been paid to the possible neurotransmitter released by the pyramidal tract (PT) neurones. From neurochemical analyses the transmitter is unlikely to be acetylcholine[4,5] or a monoamine such as noradrenaline[6,7].

Studies have therefore been carried out using the amino-acid antagonist 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) to determine whether an excitatory amino-acid could be involved.

Materials and methods. Adult male rats were anaesthetised with urethane, 1.25 g/kg. i.p. and placed on a heating pad to maintain the rectal temperature at 37–38 °C. The left cerebral cortex and right cuneate nucleus were exposed and then covered with 5% agar in saline to reduce pulsatory movements. Pyramidal tract fibres were excited by single anodal pulses of 0.1 msec duration applied to the motor or sensory areas of the cortex by a silver ball electrode. Evoked spike activity in the cuneate was considered to be monosynaptically induced, and therefore directly due to PT activity a) if the spike had a minimum latency not exceeding 5.0 msec[8]; b) if the minimum spike latency did not vary by more than $\pm$ 0.2 msec with just threshold and 5 $\times$ threshold stimuli, and c) if the spike would follow 25–100 Hz stimulation but not more than 100 Hz. These criteria were intended to eliminate from study any polysynaptically induced spikes.

Drugs were applied to single cells in the cuneate nucleus by microiontophoresis using 5-barrelled micropipettes as described elsewhere[9,10]. One barrel always contained 200 m*M* NaCl solution for current balancing and current testing[9]. The remaining 4 barrels were filled with a selection of: sodium L-glutamate 200 m*M*, pH 8.0; acetylcholine chloride 200 m*M*, pH 5.0; atropine sulphate 100 m*M*, pH 5.0; 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) 100 m*M*, pH 4.5 in 0.2 *N* HCl.

Extracellular unit activity was recorded by a single electrode fixed alongside the multibarrel assembly[10]. Spikes were amplified in a Fenlow AD55 preamplifier,

[1] Acknowledgment. I wish to thank Dr. J. .C WATKINS for the gift of a sample of HA-966.
[2] R. PORTER, Progr. Neurobiol. *1*, 1 (1970).
[3] M. WIESENDANGER, Ergebn. Physiol. *61*, 72 (1969).
[4] G. B. KOELLE, J. comp. Neurol. *100*, 211 (1954).
[5] W. FELDBERG and M. VOGT, J. Physiol., Lond. *107*, 372 (1948).
[6] A. CARLSSON, B. FALCK and N.-A. HILLARP, Acta physiol. scand. *56* suppl. 196 (1962).
[7] A. DAHLSTRÖM and K. FUXE, Acta physiol. scand. *62*, suppl. 232 (1964).
[8] T. W. STONE, Expl. Neurol. *35*, 492 (1972).
[9] T. W. STONE, J. Physiol., Lond. *225*, 485 (1972).
[10] T. W. STONE, J. Physiol., Lond. *233*, 211 (1973).

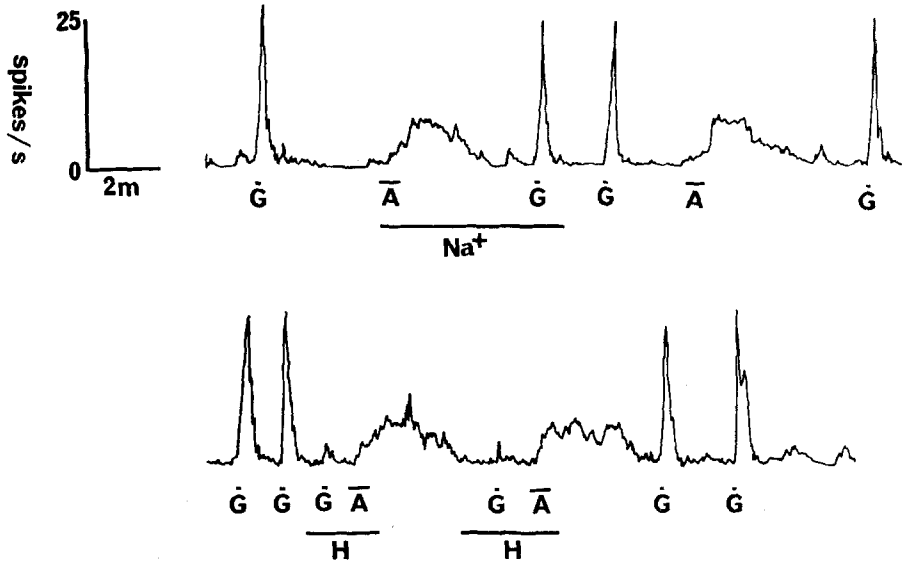

Fig. 1. Records of the firing rate of a cuneate neurone excited by glutamate, 60 nA (G) and acetylcholine, 60 nA (A). An outward current control of 100 nA (Na^+) has no effect on the cell. HA-966, 40 nA (H) abolishes the glutamate excitations without detectably affecting the acetylcholine responses. Time: 2 min.

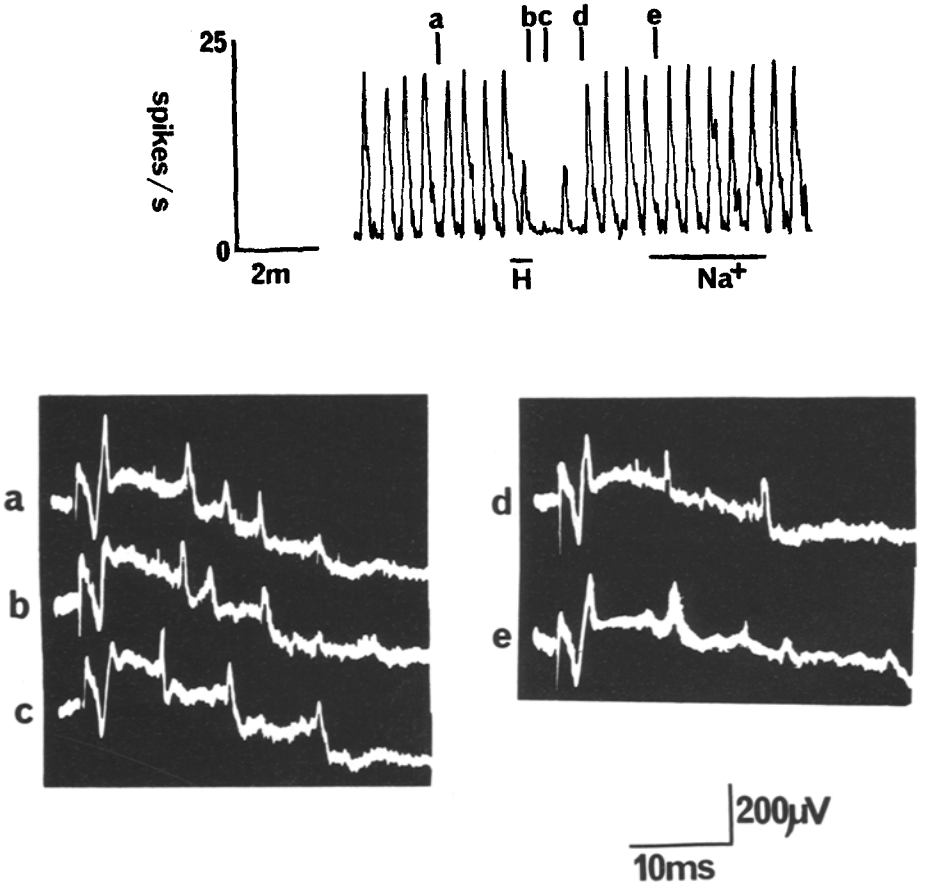

Fig. 2. Upper trace: Record of the firing rate of a neurone excited by pulses of glutamate, 60 nA. The responses are greatly reduced by HA-966, 40 nA (H), but unaffected by an outward current of 100 nA (Na^+). Lower records: Photographs of the responses of the neurone to activation of corticofugal fibres, taken at the times indicated in the upper trace. In a) is seen an early monosynaptic spike followed by polysynaptic spikes. In b) and c), taken when glutamate excitation was blocked by HA-966, the early spike has also been blocked, but the later spikes show that HA-966 was not affecting spike height at this time. The early spike reappears as the glutamate responses reappear in d). Outward current does not affect the cell (e). The spikes in d) and e) are slightly smaller than in a–c due to movement of the electrode. Upper trace: time 2 min. Lower records: calibrations 200 μV and 10 msec.

and passed through a pulse shaping and counting unit. Acontinuous record of cell firing rate was obtained on a Servoscribe pen recorder. The spike activity was also monitored on Telequipment oscilloscopes.

Results. The ability of HA-966 to antagonize amino-acid excitation was confirmed by applying the drug with an outward current of 40–60 nA to cells in the cuneate nucleus which were excited by pulses of glutamate lasting about 5 sec. HA-966 reduced glutamate excitation on 35 cells of 48 tested (73%) (Figure 1). Difficulty was experienced in demonstrating the specificity of this antagonism since no substances consistently excite cuneate neurones other than amino-acids and some chelating agents[11]. However, a few cells (6) were found which were excited by acetylcholine[11,12], and on all these cells HA-966 proved able to reduce glutamate excitation by at least 50% without affecting acetylcholine excitation to any detectable extent (Figure 1).

Monosynaptically evoked spikes were induced in 40 of 112 neurones studied in the cuneate nucleus. All but 3 of these units were encountered 600–800 μm below the surface of the cuneate nucleus. The iontophoresis of HA-966 resulted in a blockade of this monosynaptically induced activity in 28 of the 40 cells (70%). Glutamate excitation was reduced by at least 50% in all these cells at the time of synaptic blockade (Figure 2). 11 cells, including that illustrated in Figure 2, showed polysynaptically induced spikes following the monosynaptic spike. The later spikes were unaffected by HA-966, indicating that this antagonist was not having any direct local anaesthetic effect on the cell membrane, and was not producing a generalized nonspecific blockade of all synaptically induced activity. Atropine was applied to 6 cells and in none of these did any apparent change of synaptically evoked activity result.

Discussion. The efficacy and specificity of the antagonism of aminoacid excitation by HA-966[13–15] has been confirmed.

The possibility must be considered that by stimulating the cerebral cortex activity was being induced in the cuneate nucleus over pathways other than the PT. This is unlikely since it has been shown that cortically-induced excitation of cells in the dorsal column nuclei is abolished by sectioning the PT[16,17]. The PT, however, is known to send collaterals to these nuclei, capable of monosynaptically activating neurones there[18].

All the short latency spikes seen in the present study were encountered relatively deeply in the cuneate nucleus. A PT origin for these spikes is therefore supported by anatomical studies showing that PT axons terminate preferentially in the deeper layers of the dorsal column nuclei[19,20].

The antagonism by HA-966 of synaptically induced spikes therefore suggests that the neurotransmitter released by axons of the PT might be an excitatory amino-acid, though HA-966 cannot differentiate between several amino-acids such as glutamate, aspartate and DL-homocysteate[13,15]. Glutamate is a particularly strong candidate since it is present in high concentrations in the dorsal column nuclei[21], it is present in synaptosomes in the cerebral cortex[22] and it can be 'released' at the cortical surface by stimulation of some, but not all, afferent pathways[23]. It has also been shown that some cortical neurones excited by the PT are extremely sensitive to the microiontophoresis of glutamate[10]. These findings suggest that glutamate may be the pyramidal tract neurotransmitter.

[11] A. Galindo, K. Krnjević and S. Schwartz, J. Physiol., Lond. *192*, 359 (1966).
[12] F. A. Steiner and M. Meyer, Experientia *22*, 58 (1966).
[13] J. Davies and J. C. Watkins, Nature, Lond. *238*, 61 (1972).
[14] J. Davies and J. C. Watkins, Brain. Res. *59*, 311 (1973).
[15] J. Davies and J. C. Watkins, Neuropharmacology *12*, 637 (1973).
[16] S. J. Jabbur and A. L. Towe, J. Neurophysiol. *24*, 499 (1961).
[17] M. Levitt, M. Carreras, C. N. Liu and W. W. Chambers, Archo. ital. Biol. *102*, 197 (1964).
[18] F. Harris, S. J. Jabbur, R. W. Morse and A. L. Towe, Nature, Lond. *208*, 1215 (1965).
[19] F. Walberg, Brain *80*, 273 (1957).
[20] H. G. J. M. Kuypers, J. Anat. *92*, 198 (1958).
[21] J. L. Johnson and M. H. Aprison, Brain Res. *24*, 285 (1970).
[22] H. F. Bradford and A. S. Thomas, J. Neurochem. *16*, 1495 (1969).
[23] H. Jasper and I. Koyama, Electroenceph. clin. Neurophysiol. *24*, 292 (1968).

Locomotory Energetics in a Marsupial (*Antechinomys spenceri*) and a Rodent (*Notomys alexis*)

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Summary. Steady state oxygen consumption was compared in a rodent *Notomys alexis* and a marsupial *Antechinomys spenceri*. The marsupial was found to diverge from predicted eutherian energetic patterns. *N. alexis* appears to use energy storage as a significant part of the step cycle before becoming bipedal. Aerobic scope and heat storage during running are similar in both species.

Marsupials generally have standard rates of oxygen consumption about 30% below the predicted eutherian values, body temperatures 2–3 degrees lower[2] and resting heart rates about half that given for eutherian species[3]. We report here on a comparison in energy expenditure during locomotion between a marsupial and a rodent of similar body form and weight.

Materials and methods. Rates of oxygen consumption, body temperature, stride length and frequency were measured in 2 individuals of the carnivorous dasyurid marsupial *Antechinomys spenceri* (28.2 g and 31.6 g) and 5 specimens of the murid rodent *Notomys alexis* (mean body weight 27.4 g). Both inhabit the Australian desert, occurring sympatrically over part of their range. They

[1] We thank P. Baverstock, C. Watts and H. Aslin for the use of the *Antechinomys*, Ms. B. Whaite for assistance. Supported by Grants from the Nuffield Foundation, the Ingram Trust and the Flinders University Research Committee to RVB.
[2] R. E. MacMillen and J. E. Nelson, Am. J. Physiol. *217*, 1246 (1969). – T. J. Dawson and A. J. Hulbert, Am. J. Physiol. *218*, 1233 (1970).
[3] J. E. Kinnear and G. D. Brown, Nature, Lond. *215*, 1501 (1967).