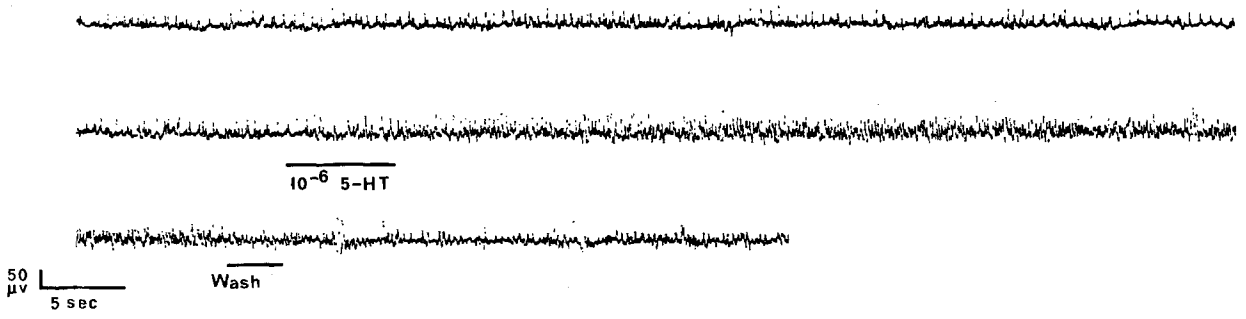

Fig. 2. Record showing the increase in muscle potential frequency caused by 5-HT (10^{-6} g/ml) applied to the muscle from the tip of a small bore pipette. The effect is reversed by washing the muscle with saline.

was the same as the latency of the antidromic potential recorded after nerve stimulation. 3. There was a one to one relationship between the GSC action potential and peripheral spike after several hundred action potentials, and after GSC firing at high frequency (Figure 1, C). The one to one relationship was not affected by bathing the preparation with saline containing high Mg^{++} and no Ca^{++}.

Individual resting lip muscles, innervated by branches of the external lip nerve, exhibited spontaneous muscle potentials which were relatively constant in frequency and amplitude. When the GSC was stimulated intracellularly to fire a burst of spikes there was a marked increase in the frequency of the muscle potentials without any noticeable change in muscle length (Figure 1, D, E). Several GSC spikes were necessary to produce this effect; no increase in electrical activity was observed after a single spike or low frequency firing from the GSC. The increase in muscle potential frequency lasted for several sec after cessation of GSC stimulation. The effect caused by GSC stimulation was abolished if the external lip nerve was severed, or if stimulating current was passed from a microelectrode placed adjacent to the GSC in the bath. The increase in electrical activity caused by GSC stimulation was mimicked by 5-HT (10^{-6} g/ml) applied locally to the muscles from the tip of a small bore pipette (Figure 2).

It is not yet clear whether the increase in electrical activity is due to 5-HT liberated from endings of the GSC directly onto the lip muscles, or whether the effect is indirect; for example via other neurons in close association with the muscles. However, fluorescence microscopy (method of FALCK[7]), bio-assay and autoradiographic experiments[8] indicate that 5-HT-containing nerve endings are present on these muscle.

The significance of the increase in electrical activity is again not yet clear, although several pieces of work appear important in relation to this phenomenon. First, TWAROG[9] has shown that 5-HT increases the occurrence of spike potentials in muscle cells of the anterior byssus retractor muscle of *Mytilus edulis* in response to nerve stimulation. Second, it has been shown by DUDEL[10] that 5-HT has an excitatory action on the crayfish neuromuscular junction by facilitating liberation of excitatory transmitter. Third, COOKE[11] has shown that exogenous 5-HT may directly facilitate neuromuscular transmission in the heart of decapod crustacea.

Finally, even if the increase in electrical activity of the lip muscles brought about by GSC stimulation is in fact indirect, it nevertheless must be considered an ultimate function of this serotonin-containing neuron.

Résumé. Le neurone géant à sérotonine (GSC) situé dans chaque ganglion métacérébral d'*Helix pomatia* envoie un axone qui se termine sur les muscles de la bouche de l'animal. La stimulation sélective du GSC provoque un accroissement significatif de l'activité électrique de ces muscles.

V. W. PENTREATH[12]

Wellcome Laboratories of Pharmacology, Gatty Marine Laboratory, The University, St. Andrews (Scotland, Great Britain), 30 October 1972.

[7] B FALCK and CH. OWMAN, Acta. Univ. lund., Sect. II, 7, 1 (1965).
[8] V. W. PENTREATH and G. A. COTTRELL, Nature, Lond. *239*, 213 (1972).
[9] B. M. TWAROG, Life Sci. *5*, 1201 (1966).
[10] J. DUDEL, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *249*, 515 (1965).
[11] I. M. COOKE, Am. Zoologist *6*, 107 (1966).
[12] I thank Dr. G. A. COTTRELL for helpful suggestions.

Strychnine and Inhibition of Bulbar Reticular Neurones

It has been shown that strychnine, an antagonist of spinal postsynaptic inhibition[1,2], reversibly blocks the action of glycine on spinal[3,4] and on bulbar reticular neurones[5]. Furthermore biochemical[6,7], and electrophysiological investigations[3,5,8,9] strongly suggest an inhibitory transmitter role for glycine in the spinal cord and medulla oblongata. An important criterium to identify a substance as an inhibitory transmitter is the demonstration that an antagonist of the depression by the artificially administered suspected transmitter also blocks synaptically induced inhibition on the same neurone. In the present study the action of strychnine on the depression by glycine as well as on synaptic inhibition produced by peripheral nerve and cutaneous stimulation of neurones of the cat brain stem has been investigated.

Recordings were obtained from neurones of the medullary reticular formation of unanesthetized, decerebrate cats. The methods have been described previously in

more detail[5]. Action potentials were recorded extracellularly through the 3 *M* NaCl-containing barrel of 4–6 barrel micropipettes of 4–6 μm tip diameter. Glycine (Fluka, 0.5 *M*, pH 3.5, HCl) and strychnine (BDH, 10 m*M* in 165 m*M* NaCl) were administered microelectrophoretically. For the stimulation of peripheral nerves (N. ischiadicus and N. radialis superficialis), bipolar platinum electrodes were used. For spinal cord stimulation concentric bipolar electrodes were inserted into the ventral part of the spinal cord at the level of the first and second cervical segment. Electrical stimuli (0,05–0,3 msec duration, 3–20 V) were generated by a Grass S 8 stimulator.

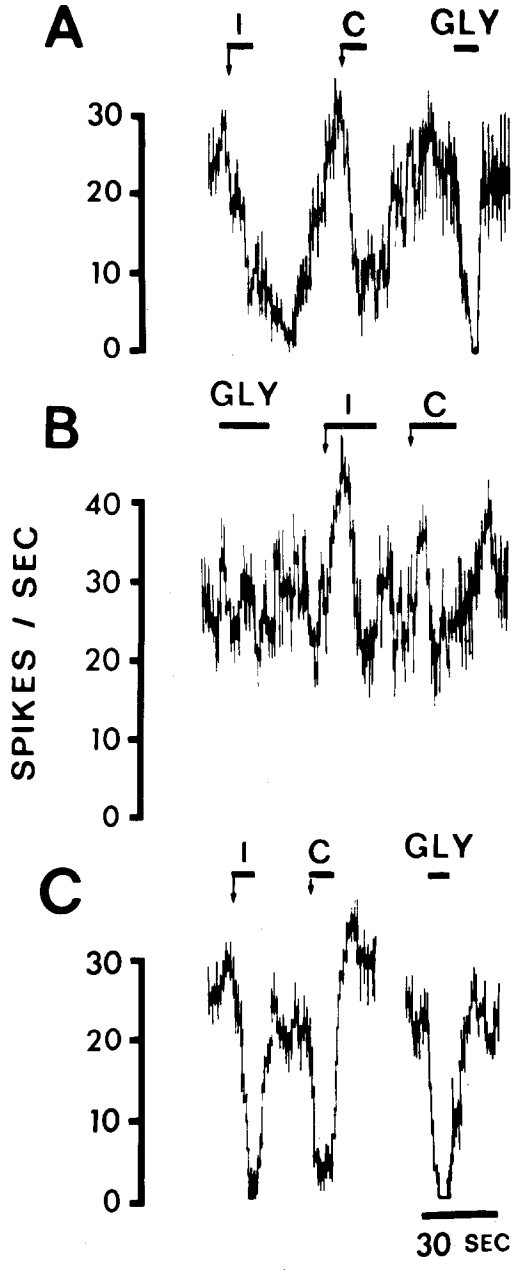

Fig. 1. Effects of strychnine on the depressant action of glycine (GLY) and the inhibition produced by squeezing the hind paws (ipsilateral I, contralateral C) on a brain stem neurone. A: before, B: during and C: after administration of strychnine 60 nA. The gap in trace C represents 90 sec. Ordinate, firing frequency in spikes/sec. Abscissa, time (30 sec).

Responses were also evoked by pinching and squeezing the limbs, trunk and tail of the animal with toothed forceps.

It has been shown by several authors that inhibition of reticular neurones can be evoked by cutaneous and/or by electrical stimulation of the spinal cord and peripheral nerves[10–13]. In the present study a comparison was made of the action of microelectrophoretically administered strychnine on synaptically induced inhibition and on the depression produced by glycine on bulbar reticular neurones. Strychnine reversibly blocked the inhibition evoked by squeezing the limbs and body and the depression by glycine on most neurones tested. An example of such an experiment is illustrated in Figure 1. Squeezing the ipsilateral and contralateral hind paws with toothed forceps markedly reduced the spontaneous firing of a brain stem neurone (Figure 1A). Glycine administered with a current of 10 nA caused an approximately equal depression of firing. Strychnine, which was ejected with a current of 60 nA for 310 sec, blocked the depression of glycine as well as the squeeze inhibition (Figure 1B) without affecting the action of GABA (not illustrated). During the administration of strychnine, squeezing the ipsilateral hind paw caused a short lasting excitation. Recovery of the squeeze inhibition and the effect of glycine was observed 6 and 10 min respectively after termination of the ejection of strychnine (Figure 1C). The difference in time course of antagonism of the action of glycine and synaptic inhibition which was usually observed might be explained by different local concentrations of glycine and the inhibitory transmitter released from the activated synapses[3].

On a small number of cells the effect of strychnine on the depressant action of taurine and synaptic inhibition was also studied. Similarly to the antagonism with glycine, strychnine reversibly blocked the action of taurine and synaptic inhibition suggesting that taurine could also act as an inhibitory transmitter at strychnine-sensitive synapses in the medulla oblongata[14].

The action of strychnine was studied on 17 spontaneously firing neurones which were inhibited by electrical stimulation of limb nerves and/or of the ipsilateral spinal cord (at the level of C1–2). A typical example of the action of strychnine on this type of inhibition is illustrated in Figure 2. The inhibitions produced by stimulation of the ipsilateral N. ischiadicus (A–C) and spinal cord (D–F) of two different brain stem neurones are reversibly blocked by strychnine (B, E) ejected with a current of 60 nA. Recovery of the antagonism of strychnine was observed

[1] K. Bradley, D. M. Easton and J. C. Eccles, J. Physiol., Lond. *122*, 474 (1953).
[2] D. R. Curtis and J. M. Crawford, A.Rev. Pharmac. *9*, 209 (1969).
[3] D. R. Curtis, L. Hösli, G. A. R. Johnston and I. H. Johnston, Expl. Brain Res. *5*, 235 (1968).
[4] D. R. Curtis, L. Hösli and G. A. R. Johnston, Expl. Brain Res. *6*, 1 (1968).
[5] L. Hösli and A. K. Tebēcis, Expl. Brain Res. *11*, 111 (1970).
[6] G. A. R. Johnston and L. L. Iversen, J. Neurochem. *18*, 1951 (1971).
[7] D. R. Curtis and G. A. R. Johnston, *Handbook of Neurochemistry* (Plenum Press, New York 1970), vol. 4, p. 115.
[8] L. Hösli and H. L. Haas, Experientia *28*, 1057 (1972).
[9] R. Werman, R. A. Davidoff and M. H. Aprison, J. Neurophysiol. *31*, 81 (1968).
[10] O. Pompeiano and J. E. Swett, Archo ital. Biol. *101*, 552 (1963).
[11] J. H. Wolstencroft, J. Physiol., Lond. *174*, 91 (1964).
[12] F. Magni and W. D. Willis, Archo ital. Biol. *102*, 434 (1964).
[13] M. Ito, M. Udo and N. Mano, J. Neurophysiol. *33*, 210 (1970).
[14] H. L. Haas and L. Hösli, Brain Res. *52*, 399 (1973).

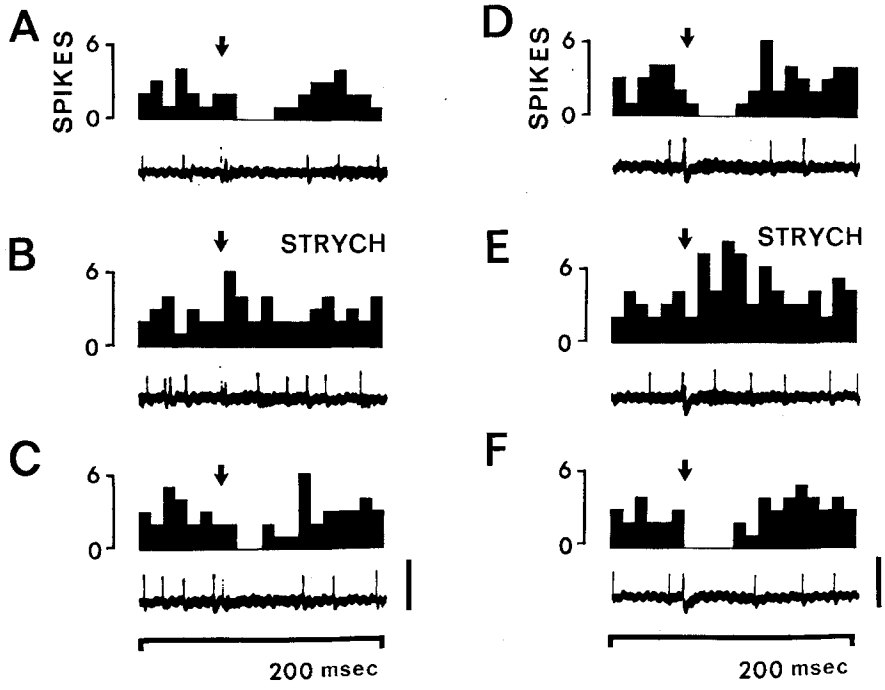

Fig. 2. Effects of strychnine on inhibition produced by electrical stimulation of the ipsilateral ischiadic nerve (A–C) and of the ipsilateral spinal cord (D–F) of two different brain stem neurones. D–F: Reticulospinal neurone. A, D: before, B, E: during and C, F: after ejection of strychnine 60 nA. Histograms summing the number of spikes of 10 sweeps (antidromic spike not counted). Oscilloscope trace of one sweep is illustrated below each histogram. (Calibration 1 mV). Time of the stimulus is indicated by arrow. Ordinates, number of spikes. Abscissae, time (200 msec).

approximately 4 min (Figure 2C) and 3 min (Figure 2F) after terminating the ejection of the convulsant. On the same cells the depressant action of glycine was also antagonized by strychnine (not illustrated). Similar results have been obtained on 3 other reticulospinal neurones which were identified by antidromic stimulation from the spinal cord[15].

On three cells where strychnine (30–80 nA) clearly blocked synaptic inhibition bicuculline (5 m*M*) ejected with currents of 80–200 nA for 4–6 min had no effect on this inhibition. Preliminary intracellular studies showed that the inhibition of firing produced by peripheral nerve stimulation was accompanied by a membrane hyperpolarization. In one neurone (membrane potential – 50 mV) it was observed that an i.v. injection of 0.2 mg/kg strychnine blocked the inhibition of spike discharge and the associated hyperpolarization evoked by stimulation of the superficial radial nerve.

Our results clearly demonstrate that inhibition of medullary reticular neurones by afferent volleys produced by squeezing the paws and by electrical stimulation of limb nerves is reversibly blocked by strychnine which also antagonizes the depressant action of glycine. Synaptic inhibition of medullary reticular neurones evoked by stimulation of the pontine reticular formation and spinal cord as well as inhibition of hypoglossal motoneurones evoked by glossopharyngeal nerve stimulation have also been shown to be antagonized by strychnine but not by bicuculline[16,17]. Although the experiments of TEBĒCIS and DI MARIA[16] and our observations suggest that synaptic inhibition on bulbar reticular neurones is strychnine-sensitive, they do not exclude that there are other types of inhibition in this structure, especially since inhibition in other areas of the medulla oblongata have been found to be antagonized by picrotoxin[18,19] or by bicuculline[20].

Our observation that strychnine blocks synaptic inhibition and the depressant action of glycine on bulbar reticular neurones in a similar fashion strongly supports the hypothesis that glycine is an inhibitory transmitter at strychnine-sensitive synapses in the medulla oblongata[5,8].

Zusammenfassung. Mikroelektrophoretisch verabreichtes Strychnin blockiert in reversibler Weise die synaptische und die durch Glycin erzeugte Hemmung an Hirnstammneuronen der Katze. Aus diesen Befunden geht hervor, dass Glycin wahrscheinlich als hemmende Überträgersubstanz an strychnin-sensitiven Synapsen in der Medulla oblongata wirkt.

H. L. HAAS and L. HÖSLI[21]

Department of Neurophysiology, Neurological Clinic of the University of Basel, Socinstrasse 55 CH–4051 Basel (Switzerland),
2 March 1973.

[15] L. HÖSLI, A. K. TEBĒCIS and H. P. SCHÖNWETTER, Brain Res. *25*, 357 (1971).
[16] A. K. TEBĒCIS and A. DI MARIA, Brain Res. *40*, 373 (1972).
[17] T. J. BISCOE, A. W. DUGGAN and D. LODGE, J. Physiol., Lond. *226*, 71P (1972).
[18] A. GALINDO, Brain Res. *14*, 763 (1969).
[19] T. MORIMOTO and Y. KAWAMURA, Expl. Neurol. *37*, 188 (1972).
[20] J. S. KELLY and L. P. RENAUD, Nature, Lond. *232*, 25 (1971).
[21] We are grateful to Miss D. POMS for typing the manuscript.