Table II. Net fluid transport (ΔV) in jejunum and ileum. Effect of various poisoning

	Jejunum		Ileum	
	ΔV (mlg^{-1}h^{-1}) Mean ± SEM	$(\Delta V_2-\Delta V_1/\Delta V_1)\times 100$ Mean ± SEM	ΔV(mlg^{-1}h^{-1}) Mean ± SEM	$(\Delta V_2-\Delta V_1/\Delta V_1)\times 100$ Mean ± SEM
Control	0.629 ± 0.151 (14)		0.800 ± 0.146 (15)	
Ouabain $10^{-4}M$	0.267 ± 0.087 (9)	−54.2 ± 6.0	0.099 ± 0.024 (10)	−82.8 ± 5.1
Ouabain $5\times10^{-4}M$	0.331 ± 0.111 (8)	−53.3 ± 9.5	0.089 ± 0.046 (11)	−92.3 ± 2.0
DNP $5\times10^{-4}M$	0.016 ± 0.014 (7)	−95.5 ± 4.5	0.039 ± 0.014 (7)	−88.0 ± 9.1

Transport values are referred to 1 g dry weight. Number of experiments in parantheses. ΔV_1 represent the control value; ΔV_2 represents the experimental value after drug addition.

In both intestinal tracts net fluid transport is completely inhibited by DNP 5×10^{-4} M while ouabain causes a similar decrease in the ileum. In jejunum, on the contrary, only a 50% inhibition in net fluid transfer is reached (Table II). It seems therefore that net fluid transport in rabbit jejunum is at least partly dependent on a Na-K ATPase system which is insensitive to the drug.

Moreover the results reported on chloride fluxes suggest that the chloride active transport process is, in both intestinal tracts, dependent on another ATPase system which is also not ouabain sensitive.

Riassunto. È stato studiato l'effetto della ouabaina sul trasporto di Na, Cl e fluido in digiuno e ileo isolato di coniglio. Mentre in entrambi i tratti intestinali il flusso mucosa-serosa di Na è inibito dal glicoside, il flusso nello stesso senso di Cl, di cui è stata dimostrata una componente attiva, non viene modificato. Poichè inoltre il trasporto di fluido viene inibito dalla ouabaina in misura diversa in digiuno e in ileo, si suggerisce la presenza di sistemi ATPasici non ouabaino-sensibili accanto alla Na-K ATPasi sensibile alla ouabaina che si ammette essere responsabile del trasporto di Na.

B. GIORDANA, F. REPETTO and A. BIANCHI

Istituto di Fisiologia Generale dell' Università di Milano, via Mangiagalli 32, I-20133 Milano (Italy),
2 May 1973.

Ionic Mechanisms Underlying the Depolarization of L-Glutamate on Rat and Human Spinal Neurones in Tissue Culture

Biochemical and electrophysiological studies have provided much evidence that glutamate may function as excitatory transmitter in the mammalian spinal cord (for ref. see[1,2]). Glutamate which is present in high concentrations in the dorsal roots[3,4] has been found to be taken up by a high affinity transport system into synaptosomes and slices of rat spinal cord[5]. Autoradiographic studies on rat and human spinal cord cultures have demonstrated that L-^{3}H-glutamic acid is taken up in neurones as well as in glial cells[6]. Furthermore, it has been shown that microelectrophoretically administered glutamate caused a depolarization of spinal motoneurones of the cat[1,7]. There is, however, little evidence relating to the ionic mechanisms that are responsible for this depolarization. In the present investigation we have used the technique of tissue culture to study ionic mechanisms underlying the depolarizing action of glutamate on rat and human spinal neurones.

Explants from spinal cord of human fetuses (8–18 weeks in utero) and of newborn and fetal rats (18 days in utero) were grown on collagen-coated coverslips for 11–40 days in vitro in the Maximov assembly (for details see[8]).

Intracellular recordings were made with glass microelectrodes (tip diameter $<$ 1 μm) filled with 3 M KCl, 2 M K-citrate or 1 M K-acetate by the method described by TASAKI et al.[9]. The microelectrodes were introduced by micromanipulators from above into the cultures, which were placed in a perfusion chamber[10] mounted on a reverse microscope. For more detailed description see[11,12].

The recording electrode was connected through an Ag-AgCl wire to a cathode follower and potentials were displayed on an oscilloscope and on a rectilinear ink recorder. The temperature of the perfusion solution was

[1] D. R. CURTIS, A. W. DUGGAN, D. FELIX, G. A. R. JOHNSTON, A. K. TEBÈCIS and J. C. WATKINS, Brain Res. *41*, 283 (1972).
[2] J. L. JOHNSON, Brain Res. *37*, 1 (1972).
[3] L. T. GRAHAM, R. P. SHANK, R. WERMAN and M. H. APRISON, J. Neurochem. *14*, 465 (1967).
[4] A. W. DUGGAN and G. A. R. JOHNSTON, J. Neurochem. *17*, 1205 (1970).
[5] W. J. LOGAN and S. H. SNYDER, Brain Res. *42*, 413 (1972).
[6] L. HÖSLI, E. HÖSLI and P. F. ANDRÈS, Symposium on Dynamic Aspects of the Synapse, Boldern, Zürich. Brain Res. *62*, in press (1973).
[7] G. BERNARDI, W. ZIEGLGÄNSBERGER A. HERZ and E. A. PUIL Brain Res. *39*, 523 (1972).
[8] E. HÖSLI and L. HÖSLI, Brain Res. *30*, 193 (1971).
[9] I. TASAKI, E. H. POLLEY and F. ORREGO, J. Neurophysiol. *17*, 454 (1954).
[10] P. F. ANDRÈS and L. HÖSLI, Microscopica Acta *73*, 38 (1972).
[11] L. HÖSLI, P. F. ANDRÈS and E. HÖSLI, Brain Res. *34*, 399 (1971).
[12] L. HÖSLI, P. F. ANDRÈS and E. HÖSLI, Pflügers Arch. *333*, 362 (1972).

36 °C ± 1 °C and the pH was kept at 7.2–7.4 by introducing 5 % CO_2/ 95 % O_2 into the bathing fluid. Composition of the bathing fluid (Gey's balanced salt solution) was (m*M*): NaCl 137, KCl 5, $CaCl_2$ 2.4, $MgCl_2$ 2.2, Na_2HPO_4 1.0, KH_2PO_4 0.18, $NaHCO_3$ 2.9, glucose 11.1. Sodium-free solutions were made by replacing the sodium ions by choline chloride and *tris*-buffer (pH 7.4). The rate of perfusion was 12 ml/min and the volume of the chamber 2.4 ml. L-glutamate (pH 8, 0,5 *M*) was administered microelectrophoretically by means of 4-barrel micropipettes which were placed close to the cell membrane (Figure 1 A) or added to the bathing fluid in concentrations of 10^{-3} to 10^{-6} *M*.

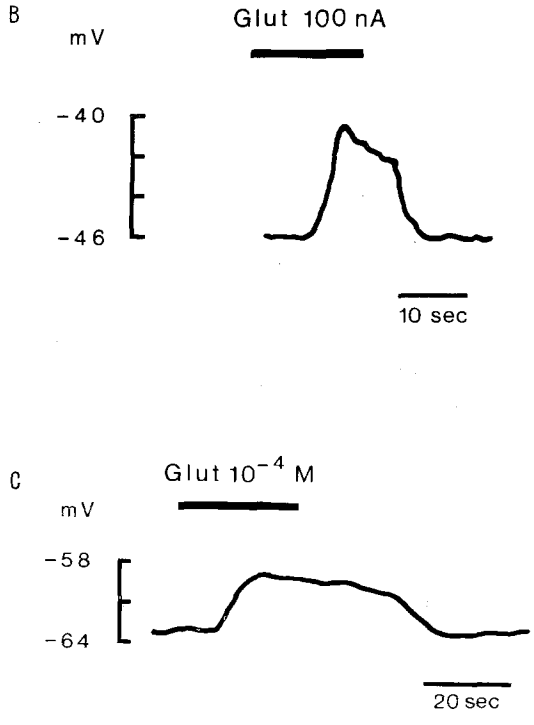

Fig. 1. A) Phase contrast picture of a rat spinal neurone in tissue culture (23 days in vitro). R, recording microelectrode; M, 4-barrel micropipette for the microelectrophoretic administration of L-glutamate. Bar: 30 µm. B, C) Effects of glutamate on the membrane potential of 2 different rat spinal neurones in tissue culture. In B) glutamate (Glut) was administered microelectrophoretically with a current of 100 nA by means of a 4-barrel micropipette. Culture 25 days in vitro. Time: 10 sec. C) Depolarization by glutamate added to the bathing fluid at a concentration of 10^{-4} *M* (Glut. 10^{-4} *M*) of another spinal neurone. Culture 26 days in vitro. Time: 20 sec. Duration of drug application is indicated by horizontal bar above tracings. Ordinate: membrane potential in mV.

The majority of microelectrode impalements were made of neurones lying in or at the edge of the explant of spinal cord cultures and only a relatively small number of recordings were made from isolated cells located in the outgrowth zones.

L-glutamate administered microelectrophoretically with currents of 100–400 nA from the tip of multibarrel micropipettes (Figure 1 A) usually caused a depolarization of cultured rat spinal neurones. An example of such an experiment is illustrated in Figure 1 B. Glutamate administered with a current of 100 nA from a 4-barrel micropipette caused a depolarization of approximately 5 mV on this neurone. As was already observed with the hyperpolarization by glycine on cultured rat spinal neurones[11] the time course and amplitude of the glutamate induced depolarization often varied considerably between individual cells.

In order to study the effects of glutamate on the membrane potential in defined concentrations, the amino acid (10^{-3} to 10^{-6} *M*) was added to the perfusing solution. (Figures 1C and 2A) Although there were sometimes considerable differences in the amplitude of the depolarizations between individual cells when using the same concentrations of glutamate, there was a clear dose-response relationship between the magnitude of effects and the concentration of glutamate. With glutamate concentrations of 10^{-3} and 10^{-4} *M* depolarizations of up to 30 mV were recorded (Figure 2A) whereas concentrations of 10^{-6}*M* usually caused only small depolarizations (1–3 mV). A few insensitive cells were also observed.

The observation that glutamate depolarized neurones in the explant of the culture as well as isolated neurones in the outgrowth zone suggests that both types of cells have glutamate receptors although electronmicroscopic studies[13] have shown that neurones in the outgrowth zone have only very few or no synapses. However, a considerable number of synapses has been found on neurones in and at the edge of the explant where most of our recordings were made.

On 4 cells the action of glutamate (10^{-4} *M*) was tested on the amplitude of hyperpolarizing square pulses passed through the recording electrode. As was observed in spinal motoneurones in situ[7] there was a decrease in membrane resistance by glutamate although only to a small extent.

There is evidence from recent studies by TAKEUCHI and ONODERA[14] at the crayfish neuromuscular junction that the currents producing the excitatory junctional potential and the glutamate depolarization are mainly carried by sodium ions. In the present investigation we have studied the effect of altering the external concentration of sodium ions on the response of glutamate on cultured human and rat spinal neurones. Removal of sodium ions from the bathing fluid reversibly reduced or abolished the depolarization by glutamate on 17 neurones tested. An example of such an experiment is illustrated in Figure 2 on a human spinal neurone. Glutamate (10^{-4}*M*) had no

[13] J. R. WOLFF, E. HÖSLI and L. HÖSLI, in preparation.
[14] A. TAKEUCHI and K. ONODERA, Nature New Biol. *242*, 124 (1973).

effect on the membrane potential of this neurone in sodium-free (Na^+-free) bathing solution. During perfusion with normal bathing fluid (Na^+ 137 mM) glutamate caused a marked depolarization of the cell membrane. Returning to sodium-free solution, glutamate again had almost no depolarizing action. On some cells the depolarization by glutamate was not completely abolished after removing sodium ions. The remaining small depolarization observed in sodium-free solution (see also Figure 2A, 3rd glutamate application) could be due to the presence of some residual sodium ions around the preparation as suggested by KERKUT et al.[15] or to the possibility that a small part of glutamate current could be carried by calcium ions as reported by TAKEUCHI and ONODERA[14] at the crayfish neuromuscular junction.

Our results suggest that the depolarizing action of glutamate on mammalian spinal neurones is mainly dependent on an increase of the membrane permeability to sodium ions. Furthermore, they show that the technique of tissue culture provides a unique possibility to study ionic mechanisms underlying transmitter actions by altering the extracellular ion concentration on neurones of the mammalian central nervous system. The observations also indicate that glutamate, which is likely to be an excitatory transmitter in the spinal cord, has a similar action on cultured human as well as on rat and cat spinal neurones in vitro and in vivo[1,7].

[15] G. A. KERKUT, R. M. PITMAN and R. J. WALKER, Comp. Biochem. Physiol. *31*, 611 (1969).

[16] For details on the effects of different solutions on the tip potential see: R. H. ADRIAN, J. Physiol., Lond. *133*, 631 (1956).

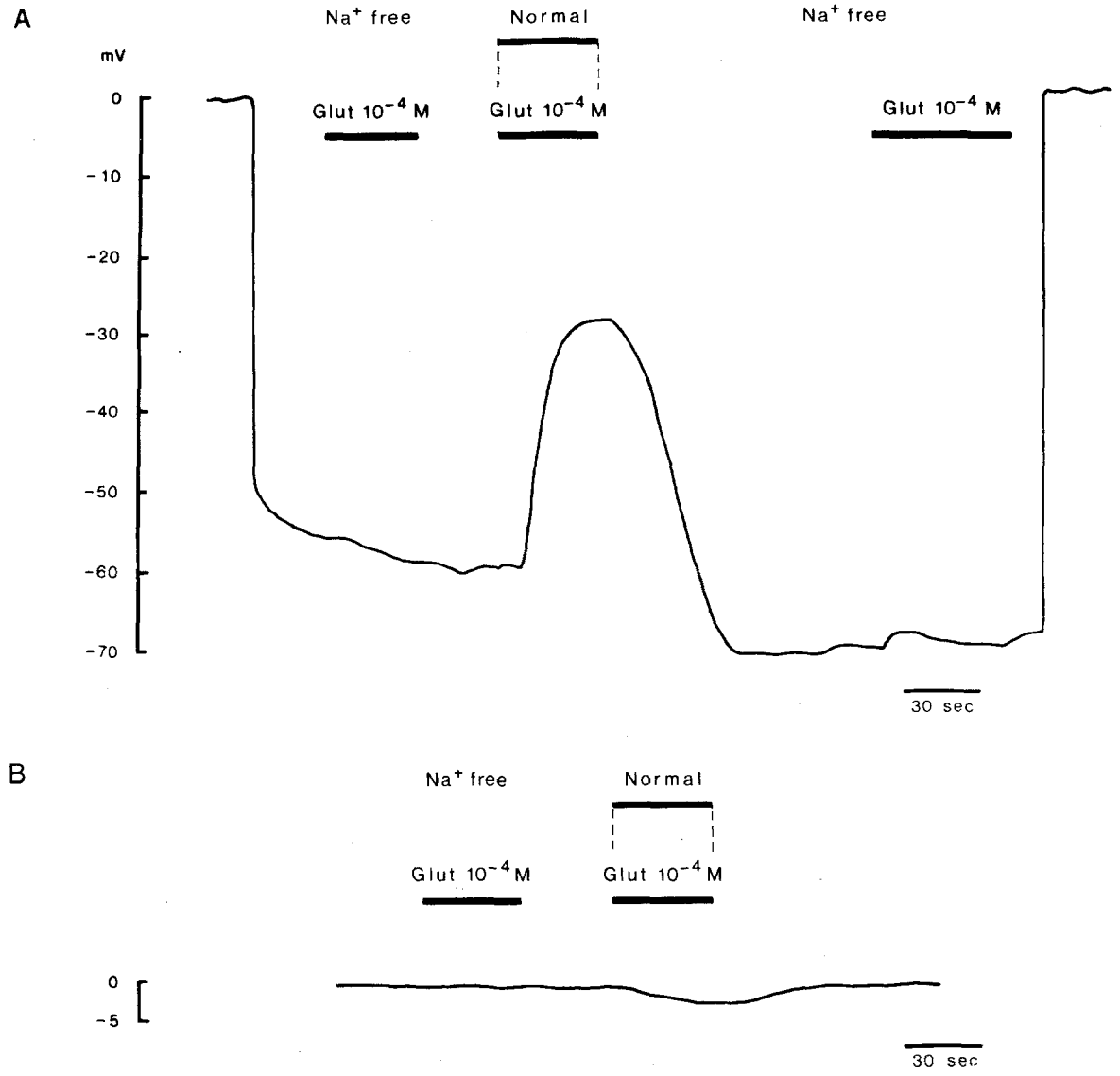

Fig. 2. Effects of removing external sodium ions on the response to glutamate (Glut 10^{-4} M) on a human spinal neurone in tissue culture (16 days in vitro, fetus 9 weeks in utero). Perfusion with sodium-free (Na^+-free) solution was started approximately 3 min before impalement of the cell by the microelectrode (1 M K-acetate). A) Effects of glutamate (10^{-4} M) on the membrane potential in sodium-free (Na^+-free) and in normal (137 mM Na^+) bathing solution. The progressive increase in membrane potential is probably due to a 'sealing-in' of the recording electrode. B) Effects of normal and sodium-free bathing solutions were tested after withdrawal of the recording electrode from the cell[16]. Ordinate: membrane potential in mV. Time: 30 sec.

Zusammenfassung. Die Wirkung von Glutamat wurde auf das Membranpotential von Rückenmarkneuronen des Menschen und der Ratte in Gewebekultur untersucht. Entfernung der Natriumionen aus der extrazellulären Flüssigkeit führt zu einem Verschwinden der durch Glutamat erzeugten Depolarisation. Diese Befunde weisen darauf hin, dass Glutamat, welches eine vermutliche Überträgersubstanz im Rückenmark ist, die Permeabilität der Neuronenmembran für Natriumionen erhöht. Die Versuche zeigen ferner, dass die Gewebekultur ein ausgezeichnetes Modell ist zur Abklärung von ionalen Mechanismen, welche der Wirkung von Überträgersubstanzen im Zentralnervensystem zugrunde liegen.

L. Hösli, P. F. Andrès and Elisabeth Hösli

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

Decrease in the Sympatho-Inhibitory Action of Clonidine after Destruction of the Sympatho-Inhibitory Area

There is considerable evidence for a centrally mediated decrease in the sympathetic tone by clonidine[1–6]. The main site of action has been localized in the medulla oblongata by transection experiments[7,8] and injection into the cephalic arteries [3,9,11]. Piperoxan [10,11] and yohimbine[11] antagonized this effect. Therefore an activation of central α adrenoreceptors has been proposed[10,11] as the cause of the sympatho-inhibitory effect. A noradrenergic mechanism inhibiting the sympathetic tone has been suggested to be localized in the medulla oblongata[10,11].

Clonidine has a much less reducing effect on the increase in the sympathetic tone brought about by central stimulation than it has on the spontaneous sympathetic tone[5,12]. Clonidine reduced the effect of submaximal stimulations but did not change or even increase the effects of supra-maximal stimulations. Waite[12] hypothesized a summation of the effects of clonidine on central mechanisms with the influence of the baroreceptors fibres on central sympathetic tone. At submaximal stimulation, the influences coming from the baroreceptor pathway are also submaximal and their sympatho-inhibitory effect could summate with the effects of small doses of clonidine. For supramaximal stimulations, the sympatho-inhibitory mechanisms are maximally activated and a summation is not possible. Therefore the increase in the sympathetic tone induced by central stimulations is unopposed. On the other hand, the first synapse of the baroreceptor pathway was localized into the nucleus tractus solitarii[13,14] and the nucleus reticularis paramedialis[15], two regions where noradrenaline-containing neurons were found[16]. A noradrenergic link in this pathway was therefore proposed[10,11].

In these hypotheses, clonidine was suggested to mimic or to activate sympathoinhibitory mechanisms in the medulla oblongata. To check this supposition, destruction in the bulbar depressor area were performed and their possible influence on the sympatho-inhibitory effect of clonidine was investigated.

Methods. Cats of either sex weighing 2–3.5 kg were anaesthetized i.v. with a mixture of chloralose (0.050 g/kg) and urethane (0.250 g/kg). They were tracheotomized but allowed to breathe spontaneously. Carotid blood pressure was recorded by means of a Statham P 23 Db pressure transducer on a San'Ei Visigraph and on a beam of a cathodic oscilloscope Tektronix 502 A using the D.C. channel.

The splanchnic nerve was isolated by a paravertebral incision retroperitoneally at its exit of the diaphragm. The nerve was stripped of its sheath and a bundle of fibres was placed on a pair of platinum electrodes. The nerve was cut distally. The splanchnic discharges were amplified by a Tektronix 122 preamplifier using the frequencies between 80–1000 Hz. The discharges were exposed on the second beam of the oscilloscope. The discharges were picked up at the output of the preamplifier, fully rectified and smoothed by means of an operational amplifier using a R. C. network of a 0.2 second time constant. The smoothed discharges were exposed on a channel of the Visigraph. The amplitude of the smoothed signal was approximately proportional to the amplitude and frequencies of the input potential. The value of the smoothed signal before the administration of clonidine was taken as 100% and the zero was determined after the death of the animal.

To expose the medulla oblongata, the head of the animal was placed in a stereotaxic instrument (La Précision Cinématographique Française); the muscles of the neck were incised and reclined. The occipito-atlantoïd membrane was opened and a part of the occipital bone was removed in order to expose the floor of the 4th ventricle. The cerebellum was gently reclined. A stainless steel electrode of 0.3 mm in diameter, varnished on its whole length except for 0.5 mm at its tip,

[1] W. Kobinger, Arch. Pharmak. exp. Path. *258*, 48 (1967).
[2] W. Kobinger and A. Walland, Arch. Pharmak. exp. Path. *257* 291 (1967).
[3] R. W. Sattler and P. A. van Zwieten, Europ. J. Pharmac. *2*, 9 (1967).
[4] H. Schmitt, Hélène Schmitt, J.-R. Boissier and J.-F. Giudicelli, Europ. J. Pharmac. *2*, 147 (1967).
[5] H. Schmitt, Hélène Schmitt, J.-R. Boissier, J.-F. Giudicelli and J. Fichelle, Europ. J. Pharmac. *2*, 340 (1968).
[6] H. Klupp, F. Knappen, Y. Otsuka, I. Streller and H. Teichmann, Europ. J. Pharmac. *10*, 225 (1970).
[7] T. Hukuhara, Y. Otsuka, R. Takeda and F. Sakai, Arzneimittelforsch. *18*, 1147 (1968).
[8] H. Schmitt and Hélène Schmitt, Europ. J. Pharmac. *6*, 8 (1969).
[9] J. W. Constantine and W. K. McShane, Europ. J. Pharmac. *4*, 109 (1968).
[10] H. Schmitt, Hélène Schmitt and S. Fenard, Europ. J. Pharmac. *14*, 98 (1971).
[11] H. Schmitt, Hélène Schmitt and S. Fenard, Arzneimittelforsch., *23*, 40 (1973).
[12] R. Waite, personnal communication.
[13] W. E. Crill and D. J. Reis, Am. J. Physiol. *214*, 269 (1968).
[14] H. Seller and M. Illert, Plügers Arch. *306*, 1 (1969).
[15] M. Miura and D. J. Reis, Am. J. Physiol. *217*, 142 (1969).
[16] A. Dahlström and K. Fuxe, Acta physiol. scand. *62* Suppl. 232 (1964).