coagulation was produced by passing a direct current through the cerebellar electrode. Paraffin sections were made and stained by cresylecht violet. Electrolytic lesions were found in the anterior vermal cortex or within or around the fastigial nucleus.

Glass capillary microelectrodes filled with 3 *M* KCl were inserted into the medial anterior hypothalamus according to SAWYER's atlas[9]. Extracellular spikes were recorded from an area extending from the ventromedial nucleus to the medial preoptic area.

After spontaneous discharges were recorded for 20 sec, effects of cerebellar stimulation were examined. The stimulus was repeated 10 times at intervals of 2 sec. Then the baseline discharges were again recorded for 20 sec.

Results. In 62 units recorded, the rate of spontaneous discharge in the control stage ranged from 0.5 to 38.7 spikes/sec with an average of 8.5 spikes/sec. The cerebellar influence was excitatory in 22 units and inhibitory in 16 units. The remaining 24 units did not respond.

Effects of cerebellar stimulation were evaluated as excitatory by the following criteria: 1. Unitary spikes were evoked by stimulation. 2. The spontaneous firing rate increased by 50 to 100% of control, while cerebellar stimulation was repeated, and returned approximately to the control rate after stimulation was stopped.

Figure A is an example of excitation. In this unit a single spike was evoked in a latency of 32 msec measured from the first stimulus pulse. Figures B, C and D are dot displays of spike discharges from another unit. B and D are control records. In C it is seen that single or repetitive spikes were evoked in a latency of 10 msec upon cerebellar stimulation and that this excitatory effect was followed by a long inhibition.

Cerebellar stimulation was judged as inhibitory when the spontaneous firing pattern changed in the following way: 1. An almost complete cessation of the spontaneous discharges followed cerebellar stimulation immediately and lasted for several hundreds msec. 2. The spontaneous discharge rate decreased to less than 50% of control for a 20 sec period during cerebellar stimulation and returned approximately to the control rate after stimulation.

Record of Figure E is to exemplify the cerebellar inhibition. In this unit, spike production was suppressed for 250 msec from the beginning of cerebellar stimulation. Figures F, G and H are dot displays spike of discharges of another neuron. Suppresion of spike discharges following cerebellar stimulation (G) was judged as significant, taking the baseline discharges of F and H as control. The mean duration of cerebellar inhibition measured from the last stimulus pulse was 494 $\pm$ 176 msec (mean $\pm$ S. E., $n = 10$).

In Figure I the spontaneous discharge rates of the hypothalamic units (ordinates) are plotted against the recording depths measured from the cortical surface (abscissae): Empty circles denote excitation and filled ones inhibition. Excitation is distributed over a wide range of discharge rates, whereas inhibition occurs preferentially in neurons with low discharge rates.

Discussion. The excitation of hypothalamic neurons by vermal lobe stimulation is in accord with the findings of SAWYER et al.[7] in rabbits. They found that stimulation of the vermis caused EEG arousal in VMH and the preoptic area. Stimulation of the vermal cortex causing an increment of spontaneous discharges of hypothalamic neurons seems to contradict the current theory of Purkinje cell action. However, ITO et al.[10] recorded the late facilitatory depolarization from cells of the medullary reticular formation following stimulation of the vermal cortex. Similar potentials may be evoked in cells of the mesencephalon by stimulation of the vermal cortex. Excitatory impulses produced there may then be conducted to the hypothalamic neurons.

The cerebellar inhibitory influence on hypothalamic neurons is not likely to be due to direct inhibitory impulses arriving at these neurons. It appears to be due to a decrease or cessation of excitatory impulses to the hypothalamus[11].

Zusammenfassung. Der Einfluss elektrischer Kleinhirnreizung auf die spontane Neuronenaktivität des medialen Hypothalamus wurde untersucht. Nach Reizung des Vermis kam es zur Erhöhung und Depression der Spontanaktivität.

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Intraaxonal Iodate Inhibits Sodium Inactivation[1, 2]

Recent experiments on single Ranvier nodes undertaken with Prof. J.F.W. KEANA[3] to test the action of specific chemical reagents on ion-specific potential and time dependent pathways across the excitable membrane showed, among other results, a considerable lengthening of the action potential if KIO_3 diffused into the axon from the cut ends. Superfusion of the nodal membrane with Na-IO_3 had no striking action, either on action potentials or on Na, K and leak currents. I have now done some more experiments to check the origin of the lengthening of the action potential by intraaxonal application of IO_3 ions.

[1] Dedicated to Prof. A. VON MURALT on the occasion of his 70th birthday.
[2] Sponsored by the Deutsche Forschungsgemeinschaft Sonderforschungsbereich 38 – Membranforschung.
[3] Department of Chemistry, University of Oregon, Eugene, Oregon, USA.
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Method. Single Ranvier nodes of motor and sensory fibres of *Rana esculenta* or *Xenopus laevis* were mounted for current- and voltage clamp[4]. The chamber and the fluid used for superfusion of the node were kept at 15 °C. At the beginning of an experiment, the internodes on either side of the node under investigation were cut after having exchanged the Ringer's solution in the side pools by isotonic KCl solution. Changes of the external fluid superfusing the nodal membrane were obtained by switching to the required solution by means of an adequate stopcock.

Inside application of IO_3 was obtained[5] by exchanging the isotonic KCl solution of the two side pools containing the cut ends of the nerve fibre by an isotonic solution of 120 m*M* K and the required amount of IO_3 and Cl to make up 120 m*M*. The cut ends were at a distance of 0.5 to 1.5 mm from the node under investigation, mostly at a shorter distance in pool E (proximal) and at a longer distance in pool C (distal) owing to the additional length of the air gap[4]. During the exchange of the fluid in the

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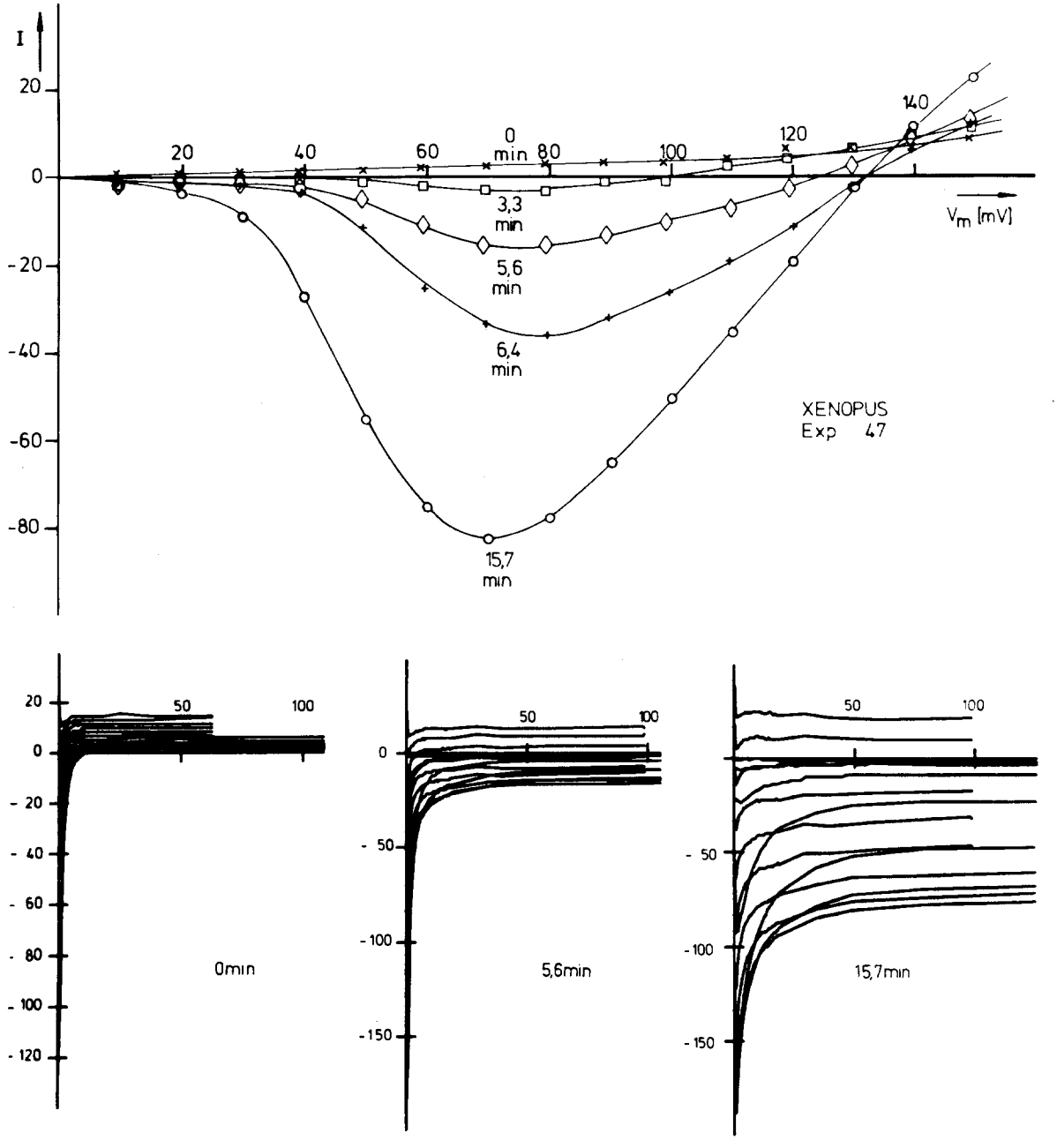

Fig. 1. Upper half: Steady state current voltage curves in TEA-Ringer before (0 min) and after replacing the isotonic KCl of the side pools by isotonic KIO_3 solution. Note increasing inward currents with a maximum at about 73 mV depolarization and zero current at about 132 mV for the two longest applications of IO_3 and probably its highest concentration reached at the internal surface of the membrane. Lower half: Clamp currents measured for diffusion times 0 (before application of IO_3) and 5.6 and 15.7 min after begin of diffusion of IO_3 into axoplasm plotted by computer. Note increase of early Na inward currents and appearance of delayed inward currents due to partial inhibition of inactivation of Na system. Abscissa: time (msec). Ordinate: current measured as potential drop along series resistance of axoplasm (mV). Distance of cut ends from node in current carrying pool 1000 µm. Exp. 47 motor fibre of *Xenopus laevis*.

side pools, the gain of the feedback amplifier was switched off and the pools were connected to ground. Each pool was washed with about 5 times its capacity with the new fluid and the overflow was sucked off to keep the fluid level constant. The procedure of changing the solution in both side pools took 30 to 60 sec. After switching back to voltage clamp, there was always a deviation of the base line of current by 1–4 mV (approximately 0.6–2.5×10^{-10} A) to the negative side (inward current) which in some of the experiments was compensated by making pool E more negative by this amount, thus bringing the base line back to zero. (The base line was recorded with an ink writer throughout the experiments.)

Action potentials were recorded on moving film to measure the change of duration and amplitude during the onset of action of IO_3 at the inner surface of the nodal membrane.

Current voltage curves for early or steady state currents in voltage clamp were obtained by using adequate on-line programs of a Honeywell DDP-516 computer giving the voltage pulses and measuring the currents. During the pause between the pulses, the current maxima were evaluated and corrected for leak currents and the curves were displayed at the end of each pulses series with automatic scaling on a storage scope; they could be photographed with a polaroid camera. More precise evaluation of the data could be obtained by storing the information on punch tape and computing the values off-line. A similar procedure was used to obtain h_∞ curves.

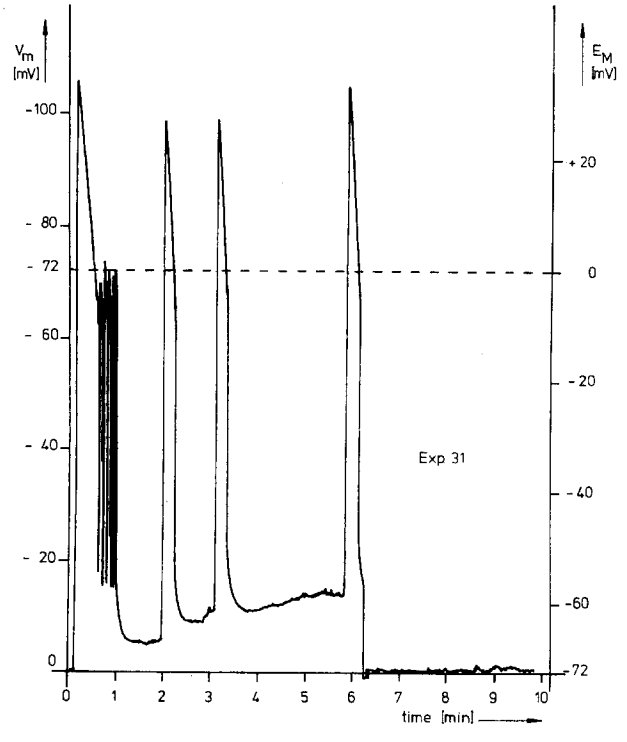

Fig. 2. Current clamp measurement of membrane potential recorded with ink writer after 19 min of application of 120 m*M* KIO_3 by diffusion into axoplasm from both side pools; motor fibre of *Rana esculenta*. 'Action potential' occuring after small depolarization of membrane followed by spontaneous action potentials. Note duration of about 1 min, oscillations, overshoot and dependence of amplitude from interval between spontaneous action potentials. After the last AP the amplifier is set to voltage clamp position and current is recorded at holding potential V = 0. The E_M scale is obtained after electrical destruction of the node indicating zero membrane potential at V = 72 mV. Exp. 31 motor fibre of *Rana esculenta*.

The absolute value of the resting potential was determined at the end of each experiment by destroying the membrane with a hyperpolarizing pulse of 360 mV in voltage clamp and by measuring the current clamp potential level which is assumed to be zero after destruction of the membrane[6].

Results. 1. If the membrane is clamped to the resting potential (70 mV approximately) in Ringer's solution, 2–4 min after exchanging the isotonic KCl of the side pools by isotonic KIO_3 solution the clamp current which had previously been set to zero begins to become inward. This inward current can be removed by using a Na^+ free external solution (*Tris*-Ringer) or by using TTX-Ringer. The trace of the ink writer used to observe changes of clamp current begins to show low frequency noise.

2. The current voltag curves for early currents at the beginning of the IO_3 induced changes show an increase of the maximum Na-currents of about 30% which then slowly vanishes.

3. The steady state I–V curves in TEA-Ringer (7.6 m*M*) develop a downward hump in the region of depolarizations around 60 mV which finally becomes a N-shaped curve resembling normal early current voltage curves for sodium crossing the voltage axis at the sodium equilibrium potential. The maximum inward current increases, the longer IO_3 is diffusing into the axoplasm and thus increasing the internal IO_3 concentration (Figure 1). The maximum early currents are usually bigger than the late steady state sodium currents – but in a few extreme cases they become equal when the total Na current begins to show a fast run down (see below).

4. Prolonged action of IO_3^- from inside after accelerated run down leads to a sudden loss of time and potential dependent sodium pathways in the membrane. Strong inward currents with an unsteady time course and much noise appear, which could be taken as indication of a membrane breakdown. However, the resistance remains high enough to compensate the strong depolarization of the membrane by hyperpolarizing currents in current clamp. The membrane, however, has lost any sign of nonlinear behaviour (in TEA-Ringer). It can be destroyed by hyperpolarizing in the usual way and the zero membrane potential level is normal, indicating an original resting potential of about 70 mV which had decreased during the action of internal IO_3^- by 10 to 30 mV.

5. Current clamp recordings of membrane potential at a stage of advanced action of internal IO_3^- in TEA-Ringer without the above-mentioned destruction of the voltage and time dependent sodium system are unstable and tend to produce spontaneous action potentials with durations of 20 to 60 sec. If the depolarization due to a permanent sodium permeability at the resting potential is overcome by a small hyperpolarizing current, the base line is stabilized and some of the low frequency noise is suppressed. The action potentials elicited under these conditions overshoot the zero potential line by 20 to 40 mV. They can be recorded with the ink writer (Figure 2) and tend to oscillate in the regenerative shut-off part of the repolarization phase.

6. h_∞ curves with internal IO_3^- are less steep and do not reach the value of complete inactivation, even at strong depolarizations (Figure 3).

7. Tail currents in TEA-Ringer's, after depolarizing pulses up to 120 mV, present an early fast and then a very slow decline as long as early Na currents are still exceeding the steady state ones.

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All the modifications mentioned are practically irreversible, even if only 2 min after exchanging the isotonic KCl by isotonic KIO_3 the side pools are again filled with isotonic KCl. Only extreme durations of action potentials may be reduced by a small percentage after returning to KCl solution.

Discussion. The results indicate that IO_3^-, which among other actions is known to oxidize cystine to 2 molecules of the corresponding sulfonic acid and to oxidize disulfide bonds in insulin[7], probably reacts with a key molecule located inside of the sodium pathway across the membrane, controlling inactivation of the sodium system. Applied to the external surface of the nodal membrane, however, IO_3^- has almost no action, except for a small increase of run down of the sodium and potassium system of the membrane[8]. This finding corroborates the view of ROJAS and ARMSTRONG[9] who, from their finding of reduced inactivation in squid axons perfused with pronase, concluded that some protein located at the inside of the excitable membrane controls inactivation of the sodium system. IO_3^-, however, bears many advantages compared to pronase. It can be applied electrophoretically through micropipettes and its mode and site of action should not be too difficult to elucidate.

Furthermore, the possibility of working with 'open' sodium 'channels' will greatly enhance the possibilities to study the action of chemical and physical agents on the sodium system with simple voltage clamp systems.

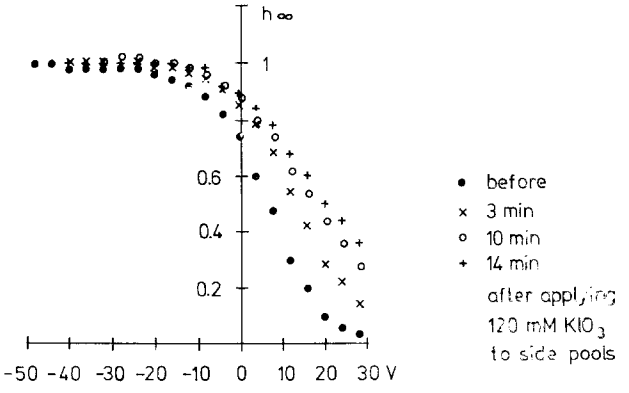

Fig. 3. h_∞ curves obtained before and after exchanging the isotonic KCl in the side pools by isotonic KIO_3. The results were obtained 'on-line' with a program developed for normal inactivation using prepulses (60 msec duration) from V = −50 to +30 mV. Note flattening of curves indicating reduced inactivation. Exp. 31 motor fibre *Rana esculenta*.

Zusammenfassung. Iodat (IO_3^-)-Ionen hemmen den Inaktivierungsvorgang des Natriumsystems, falls sie intraaxonal durch Diffusion vom abgeschnittenen Ende einer markhaltigen Froschnervenfaser her ans Innere der Schnürringmembran gelangen. Mit der Spannungsklemme erhält man dann in TEA-Ringer eine Strom-Spannungskurve des Natriumsystems auch mit lang dauernden Potentialänderungen. In der Stromklemme lassen sich unter diesen Umständen Aktionspotentiale mit Dauern bis zu 1 min registrieren.

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Muscarinic Mediation of the Biphasic Temperature Response to Intrahypothalamic Injections of Carbachol in the Cat[1]

There is considerable evidence that in several species acetylcholine may be a neurotransmitter at those synapses within the hypothalamus which are involved in the control of body temperature[2]. In the cat, injections of microgram quantities of acetylcholine or carbamylcholine (carbachol) into the anterior hypothalamic/preoptic (AH/PO) region of the brain evoke thermoregulatory changes which are dose-dependent[3]. Typically, low doses cause only a rise in body temperature and high doses, a fall. Intermediate doses produce a biphasic effect: a fall followed by a rise. To explain this dual effect on body temperature, it was postulated that the AH/PO region of the cat contains two partially overlapping cholinergic systems, one mediating heat gain and the other, heat dissipation[3]. Since both acetylcholine and carbachol possess strong muscarinic and nicotinic agonistic activity, it is possible that neurotransmission might be muscarinic in one of these systems and nicotinic in the other. The present experiments were carried out to examine this possibility.

Materials and methods. Using pentobarbital anesthesia and aseptic technic, 22 G stainless steel guide cannulae were implanted just above the AH/PO region in male cats weighing 2.5–3.7 kg. Two weeks were allowed for recovery from surgery. Through a 28 G injection cannula, sterile, pyrogen-free, isotonic solutions of drugs dissolved in an artificial cerebrospinal fluid were injected unilaterally in a volume of 1.0 µl at a depth of 1.5 mm below the guide tips.

During each experimental session, the cats were isolated in a temperature-controlled cabinet (20 °C ± 0.5 °C) and restrained in a stock-like device to which they had been previously accustomed. Colonic temperature, ear skin temperature and respiratory rate were monitored continuously throughout the session. These parameters were allowed to stabilize for at least 1 h before an intracerebral injection was performed.

The following drugs were used: carbamylcholine chloride, nicotine dihydrochloride, oxotremorine base or sesquifumarate salt, l-hyoscyamine hydrochloride and mecamylamine hydrochloride.

Results and discussion. In confirmation of a previous report[3], intrahypothalamic injections of 0.01 *M* to 0.03 *M* carbachol into AH/PO loci produced biphasic

[1] Supported in part by Grant No. NS11175-01 from the National Institutes of Health, U.S. Public Health Service.
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