Recovery of Neuronal Function after Prolonged Cerebral Ischemia

Abstract. *Cats were submitted to complete cerebral ischemia by clamping the innominate and subclavian arteries and simultaneously lowering the systemic blood pressure. Neuronal function was assessed by recording the electroencephalogram and the anti- and orthodromic activation of the pyramidal tract. A full recovery of the pyramidal response and even of evoked electroencephalographic activity occurred after ischemia of more than 1 hour's duration.*

Damage inflicted upon the brain by transient ischemia is considered to result directly from the oxygen deprivation of the neuronal elements. Since the normothermic brain cannot be revived after more than 8 to 10 minutes of complete ischemia (*1*), this time limit generally is considered to mark the "death" of most neurons. Recently it has been reported that complete cerebral ischemia of more than 7 minutes' duration is accompanied by a swelling of the vascular endothelial and perivascular glial cells (*2*). This may result in the obstruction of the vessel's lumen and in a serious impairment of the blood recirculation after ischemia ["no reflow phenomenon" (*3*)]. Thus "brain death" following a transient block of the cerebral blood supply may be caused indirectly by the "no reflow phenomenon" even when the initial ischemic impact was not fatal by itself. This assumption is supported by experiments in which we were able to maintain an adequate blood recirculation for a limited time period (*4*). In these cases a beginning recovery of neuronal function was observed after ischemia up to 16 minutes, but a secondary suppression occurred when the circulatory disorder became apparent. In the experimental model presented here, the blood recirculation remains unimpaired for an unlimited time period, and a recovery of neuronal functions occurs after ischemia of considerably longer duration.

We have used 16 normothermic cats which had been anesthetized by the intraperitoneal injection of 30 mg of Nembutal per kilogram of body weight. Cerebral ischemia was produced by interruption of the arterial blood supply leaving the venous outflow open in order to avoid stagnation of blood in the cerebral vessels. Thoracotomy was performed on the left side of each cat while it was artificially ventilated with room air. The internal mamillary artery was ligated and the innominate and subclavian arteries were clamped for periods from 20 minutes to 2 hours. During the clamping the systemic blood pressure was lowered to about 80 mm-Hg by intravenous infusion of an antihypertonic agent (trimethaphan camphor sulfonate) in order to prevent any collateral blood supply to the brain. The cessation of the cerebral blood flow was controlled by microscopic observation of pial vessels in the sensorimotor area. This was confirmed by the intravenous injection of a dye (Evans Blue), at different time intervals after the clamp, which revealed the total ischemia of the brain and spinal cord down to the upper thoracic level.

The functional impact of ischemia was assessed in the area of the sensorimotor cortex by recording the spontaneous electroencephalographic (EEG) activity and the response of the pyramidal tract (PR) to electrical stimulation of this site. The PR was elicited by a single pulse of 0.3 msec duration and recorded from the lower pyramid by a bipolar needle electrode which was placed stereotactically by the dorsal approach. This response consists of two or more waves, the first of which (D-wave) is the direct, antidromic response of the cells which give rise to the pyramidal tract. The following waves (I-waves) are the indirect orthodromic response of the pyramidal-tract neurons through interneurons which also are activated by the electrical pulse. Since the ablation of the cortex abolishes the I-waves (*5*), their occurrence must be considered as positive evidence for synaptic transmission in this area.

In our experimental situation the EEG was suppressed after 12 ± 2 sec-

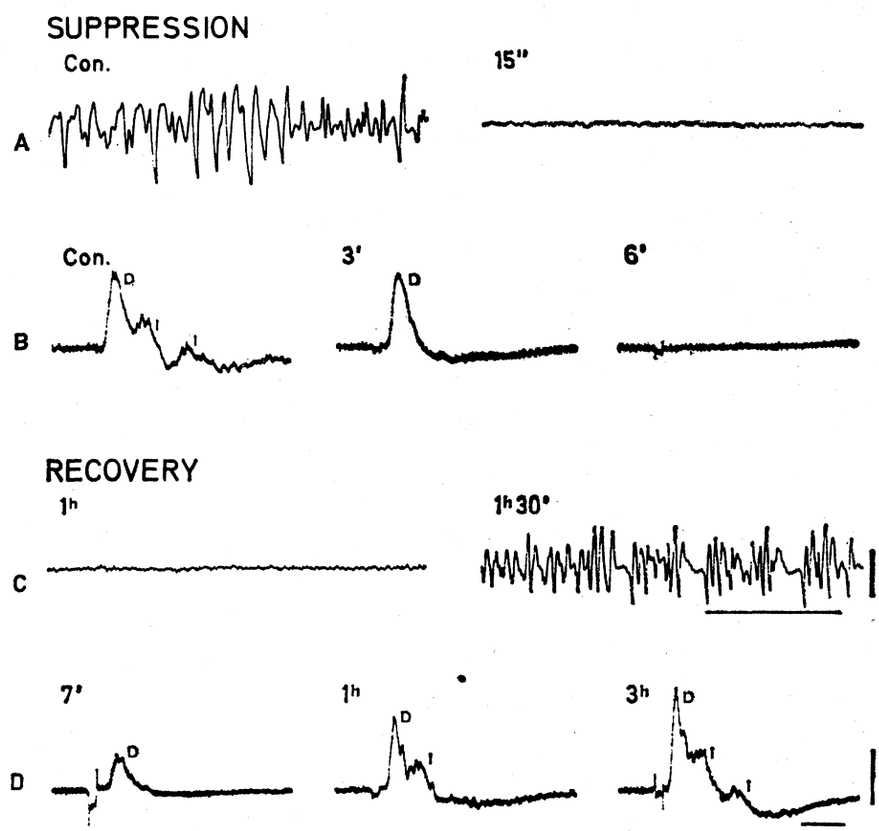

Fig. 1. Recording of the EEG and of the pyramidal response (PR) after electrical stimulation of the sensorimotor cortex during ischemia of 1 hour's duration. (A) Electroencephalogram from the right sensorimotor cortex immediately prior (*Con*) and 15 seconds after the onset of ischemia. (B) The PR prior to ischemia (*Con*) consists of a D-wave (*D*) and two I-waves (*I*). Three minutes after the onset of ischemia the I-waves are suppressed, and after 6 minutes, the D-waves also. (C) One hour after the blood flow has been restored the EEG has not reappeared, but after 1 hour and 30 minutes a burst of EEG activity can be elicited by a strong electrical stimulation of the cortex. (D) Seven minutes after the blood flow has been restored a small D-wave has already recovered. After 1 hour one I-wave, and after 3 hours two I waves, can be elicited. Calibration: EEG: 100 μv, 1 second; PR: 200 μv, 2 msec.

onds, the I-waves of the PR after 3 ± 1 minutes, and the D-wave after 4.5 ± 1.5 minutes of ischemia (Fig. 1). After the clamps were released the brains in all instances were immediately recirculated with blood, and cerebral pulsations were visible. There was also an immediate color difference between the pial veins and arteries, indicating the oxygen uptake of the brain. An impairment of the recirculation could effectively be avoided by simply raising the systemic blood pressure to 140 mm-Hg by intravenous infusion of noradrenaline after the clamps had been released.

The recovery process of neuronal function was relatively independent of the duration of ischemia and started surprisingly fast after the blood flow was restored (Fig. 1). After total ischemia of up to 1 hour, the latency of recovery of the D-wave was less than 10 minutes, and that of the I-wave was less than 30 minutes. Only the recovery of the EEG showed a certain relationship to the duration of ischemia. After ischemia of less than 30 minutes the EEG recovered within 1 hour, whereas in the longer experiments only short-lasting bursts could be elicited by a strong cortical stimulation (Fig. 1). A recovery of neuronal function after ischemia of more than 1 hour's duration was no longer predictable. In one case, however, a transient recovery of the D-wave of the PR occurred even after 2 hours of total ischemia.

Information about the permanence of recovery is still limited, since this investigation was performed in acute experiments. After ischemia of less than 40 minutes a secondary suppression of neuronal activity was not observed, although the animals were kept alive up to 24 hours. In some of the experiments in which ischemia was longer than 40 minutes, recovery was only transient and a secondary suppression which was not due to an impairment of the cerebral blood flow occurred 4 to 6 hours later. At present we have no explanation for this phenomenon.

The mechanism of the recovery process remains to be clarified. The prerequisite for any recovery, of course, is an adequate blood recirculation. However, noradrenaline which has been used to control the systemic blood pressure may have a direct effect on the cortical neurons, although such an action has been refuted in normal animals (*6*).

The fact that unequivocal signs of neuronal recovery can be detected after complete ischemia of more than 1 hour's duration raises serious questions about the reliability of criteria currently used for the determination of brain death. Furthermore, our experiments suggest that concrete chances exist for the successful treatment of prolonged cerebral ischemia.

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Reactions of Alpha Methylene Lactone Tumor Inhibitors with Model Biological Nucleophiles

Abstract. *Thiols are the most reactive nucleophilic reagents among the biological models investigated. They undergo "Michael-type" addition to the polyfunctional sesquiterpene lactones. The rapid rates of reaction with* L-*cysteine were measured and the reaction products were characterized. Each addition of thiol successively decreased the cytotoxicity of the adducts formed.*

The search for tumor inhibitors of plant origin has led to the isolation and characterization of a number of sesquiterpene lactones (*1*) that exhibit growth inhibitory activity in vivo against animal tumor systems and in vitro against cells derived from human carcinoma of the nasopharynx (KB). Many of these biologically active lactones are α-methylene γ-lactones that have other functional groups, such as epoxide, chlorohydrin, unsaturated ester, unsaturated lactone, and unsaturated ketone groups. Little is known about the relation between structure and activity in these compounds (*2*), but the demonstrated reactivity of unsaturated lactones toward thiols (*3*) and amines (*4*) and the presence of other reactive functional groups suggested that the cytotoxicity of these compounds may result from alkylation of nucleophilic centers in a biological system. Therefore the chemical reactivity of some of the cytotoxic lactones toward model biological nucleophiles is being studied and we now describe the reactions of elephantopin (**1**, *5*), eupatundin (**2**, *6*), and vernolepin (**3**, *7*) with aqueous solutions of L-cysteine at *p*H 7.4.

Second-order rate constants (Table 1) for the reactions of the lactones with cysteine were determined spectrophotometrically with a Beckman DK-2A spectrophotometer equipped with a thermostated cell compartment. To $10^{-4}M$ cysteine in $0.067M$ phosphate buffer (*p*H 7.4), prepared in a 1-cm quartz cell (volume, 3.60 ml), $10^{-2}M$ lactone in tetrahydrofuran (36 μl) was added, and the resultant solution was mixed rapidly. After an appropriate reaction time, the sulfhydryl content was measured by quenching the reaction with an excess of a tetrahydrofuran solution of 2,2′-dipyrydyl disulfide, which reacts with cysteine to give 2-thiopyridone (*8*) ($\varepsilon = 7780$ at 343 nm). The rate of addition of the second mole of cysteine to **4** was determined by allowing the reaction of an equimolar mixture of **1** and cysteine to reach completion and then measuring the rate of reaction of this preformed adduct with an added mole of cysteine. Eupatundin and vernolepin also reacted with a second mole of cysteine.

Comparison of the rate constants of the foregoing reaction with reported values for the reactions of *N*-ethylmaleimide and iodoacetate with cysteine showed that the lactones have about the same order of reactivity toward cysteine as iodoacetate does. In contrast to the reactivity of lactones **1**, **2**, and **3** toward sulfhydryl groups under these conditions, their reaction with amino groups appears to be very slow. When a solution equimolar in lysine and vernolepin (**3**, $10^{-2}M$ in 50 percent aqueous tetrahydrofuran) at *p*H 7.4 was allowed to stand for 6 days at 25°C, 75 percent of the original lactone could be recovered. Guanine also proved unreactive toward either vernolepin or elephantopin.

The cysteine addition products were isolated from a solution of $10^{-2}M$ lactone in aqueous tetrahydrofuran (*p*H