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A Method for Study of the Interrelation Between EEG and Blood-Brain Barrier Phenomena

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Abstract

FLODMARK, S. and O. STEINWALL. *A method for study of the interrelation between EEG and blood-brain barrier phenomena.* Acta physiol. scand. 1962. *56.* 112—119. — An experimental procedure has been elaborated to facilitate selective effects exerted by agents applied within the cerebral vessels on EEG and blood-brain barrier phenomena. In rabbits short-term (less than 1 minute) perfusion of one hemisphere is performed via the ipsilateral internal carotid artery with a pressure adjusted so as to obtain displacement of the blood. This displacement is controlled by inspection of the pial vessels through a trephine opening. The technique implies control of the active concentration *in loco* of the applied agents and of the application time, hence graded influences near the threshold levels can be obtained.

Structural and metabolic considerations make it conceivable that low grade damage of the blood-brain barrier may be produced with no significant effect on the neuronal activity as reflected in EEG (except for the brief influence from the blood deprivation). After such damage intravenous administration of suitable substances, unable to pass the intact barrier, may give rise to unilateral EEG changes and thus reveal the defective barrier function. Two model experiments are reported. The first one shows that unilateral blood deprivation *per se* (for about 2 min) exerts a marked but reversible effect on the EEG without damage to the blood-brain barrier as tested by intravenously injected trypan blue. The second experiment illustrates an attempt to induce barrier damage without significant changes in the EEG.

The electrical activity as observed in the EEG is essentially generated in neuronal structures and thus primarily reflects the state of the neurons. It has not been shown that the glia cells actually produce electrical phenomena of direct significance for the EEG, see TASAKI and CHANG (1958), but they no doubt exert an indirect effect on the EEG by virtue of their decisive influence on neuronal metabolism, *i. a.* see HYDÉN (1960). The interposition of glial elements between neurons and brain capillaries strongly suggests that the perivascular glia is involved in the regulation of blood-brain exchange of substances. This does not necessarily mean that the special phenomena, generally accorded to the blood-brain barrier concept are solely glial effects. This concept probably encompasses a multiplicity of mechanisms, the background of which may be found in different structures and metabolic factors, *i. a.* see BROMAN (1955), DOBBING (1961), EDSTRÖM and STEINWALL (1961), TSCHIRGI (1961).

Correlations between the EEG and blood-brain barrier phenomena have been reported by many investigators, *i. a.* PRADOS *et al.* (1945), AIRD (1949), BLOOR *et al.* (1951 b), FUNDERBURK and CASE (1951), GONSETTE (1956), PURPURA *et al.* (1958), PURPURA and CARMICHAEL (1960). The present technique for experiments on rabbits has been elaborated in order to penetrate the possibilities of inducing differentiated effects on neuronal activity and barrier functions.

During EEG recording various agents are applied within the blood vessels of one hemisphere; the other serves as a control with respect to EEG as well as to the influence of the applied agents on barrier function. Special attention has been directed to the achievement of a reliable control of the concentration *in loco* of the applied agents as well as of the application time. By that means influences near the threshold level can be obtained, and this presumably favours the occurrence of dissociated influences on either the barrier or the EEG. In the present paper the experimental procedures and two illustrative experiments will be reported and discussed.

Experimental procedures

Adult rabbits in urethane anaesthesia were used. Blood pressure was measured by means of a mercury manometer connected to a femoral artery. In some experiments the electrocardiogram and the respiratory rate were simultaneously recorded.

EEG recording. After preliminary trials with different types of electrodes chloridized silver screws, 10 mm in length, were found most suitable. The screws were applicated intraosseally, without injury to the lamina interna of the bone, because pressure lesions easily occur at epidural application even when the dura is intact. Further fixation and isolation of the electrodes was obtained with plastic cement. The electrodes were symmetrically placed over the hemispheres, anteriorly over the motor region just in front of the sutura coronaria, and posteriorly over the occipital region. The distance from the midline was about 5 mm. Bipolar recording with an eight-channel Grass EEG machine was used.

Intravasal application of agents. The application of agents within the vessels of one hemisphere was performed according to a technique developed by BROMAN and OLSSON (1948, 1956) and modified by STEINWALL (1958). The solution was injected through a plastic catheter secured in one common carotid artery after ligation of its proximal part and its external branches. The injection pressure was kept at the blood pressure level so as to obtain displacement of the blood from the ipsilateral hemisphere. This was controlled by inspecting the pial vessels through a trephine opening with the transparent dura left intact. The injection time was kept within 30—45 sec. Afterwards blood from the other arteries supplying the circle of Willis recirculated through the perfused vessels. In the event that highly toxic agents be employed a reduction of their concentration in the general circulation might be attained by drainage from the external jugular veins during the injection.

The applied chemical solutions were approximately neutral and isotonic and were filtered before injection. When the application time is kept within the narrow limits mentioned, the concentration of the noxious agents primarily determines their effect, whereas the injected volumes (usually between 5 and 10 ml) are of minor importance.

Testing the state of the blood-brain barrier. In order to ascertain whether or not the intravasal application of the agents induced inhibition of the blood-brain barrier suitable indicator substances, which are unable to pass from blood to brain under normal barrier conditions, were introduced into the general circulation. In the current experiments the indicators were chosen from among the group of anion dyes and other organic acids discussed by STEINWALL (1961). The effect on the EEG was studied as a functional indication of the state of the barrier. Furthermore the brains were scrutinized *post mortem* to reveal any staining by the intravitally applied indicator dyes. The animals were killed by exsanguination and the cerebral vessels rinsed with saline under one meter of pressure for one minute before removal of the brain.

Comment. The described procedure for intravasal application of solutions within one hemisphere involves an obvious interference with the cerebral blood supply by the occlusion of one carotid artery and the brief blood deprivation during the injection.

Occlusion of one carotid artery in rabbits brings about circulatory effects which have been thoroughly studied, see MCDONALD and POTTER (1951), JEPPSSON and OLIN (1960), KRUPP (1961). Through the well-developed circle of Willis the ipsilateral hemisphere is sufficiently supplied with blood. At ligation the systematic blood pressure may increase up to 20 per cent. KRUPP reports that occlusion of one carotid artery induces only brief bilateral changes of arousal type in the EEG. In the present series this has been confirmed. Even clamping of both carotid arteries or unilateral occlusion combined with lowering of the blood pressure by about 50 per cent (by means of trimethaphan) has caused no significant effect on the EEG in our experiments.

The deprivation of blood from one hemisphere during the carotid injection obviously gives rise to anoxic effects calling for special attention as to their influence on the EEG. By iterated controls in a number of experiments it has been found that such blood deprivation by Ringer's solution, saline, or isotonic glucose uniformly induces unilateral depression of the amplitudes and a slowing of the frequency pattern after 20 to 30 seconds. Unless the injection lasts for

more than 60 seconds the EEG changes disappear within half a minute after the injection was terminated. These transient effects thus need not disturb the interpretation of the more protracted changes derived from chemical influences, when certain noxious agents are injected.

With a technique similar to the present one BLOOR *et al.* (1951 a, 1951 b) studied EEG and blood-brain barrier effects, however, without control of the blood expulsion which, with our method, is checked by inspection through the trephine opening. In other methods of principally the same type stress has been laid upon minimizing interference with cerebral circulation. To that end the agents have been injected directly in the streaming blood of one carotid artery by means of a very thin needle (SHIMIZU *et al.* 1952) or introduced via a catheter secured in a branch opening into the carotid trunk (MCDONALD and POTTER 1951, ROTHBALLER and JARVIK 1958, ROVIT *et al.* 1960). All these methods obviously permit distinct unilateral influences but they are not designed to yield control as to the concentration of the applied agents *in loco.*

Urethane anesthesia has been employed in most experiments. This drug was chosen because it induces a continuous anesthesia at a level where alerting effects may easily be obtained by external stimuli. These variations in alertness often enhance the asymmetric EEG changes sought. In our opinion this type of anesthesia has no inherent draw-backs for the current experiments.

Model experiments

1. *Effects of unilateral blood deprivation*

An adult rabbit weighing 2.2 kg in urethane anesthesia was used. Registration of the blood pressure reaction at temporary clamping of the carotid arteries showed an elevation of 5—10 mm from 90 mm Hg.

Symmetric EEG records were obtained before and after catheterization of the left carotid artery. Ringer's solution (with glucose to 0.25 %) was injected twice through the carotid catheter causing blood expulsion from the left hemisphere. The first injection (20 ml) lasted for 55 sec. In the EEG depressed amplitudes and an increase of slow waves appeared 20 sec after the start of the injection. These changes were limited to the left side and disappeared promptly when the injection ceased. Ten minutes later a second injection (40 ml) was administered for a period of 2 min (Fig. 1 A, B). About half a minute after the start the same type of left-sided EEG-changes commenced and gradually increased, giving rise to a pronounced asymmetry with very slow waves (1—2 c/s) while the right side showed arousal effects. At termination of the injection distinct changes on the left side persisted for about one minute and a slight asymmetry was visible for at least another minute. An i.v. injection of 18 ml trypan blue (1 %) was started 20 sec after the end of the carotid injection (Fig. 1 C). The EEG during the remaining 15 min of the experiment was symmetric and without any noteworthy features (Fig. 1 D). After exsanguination and

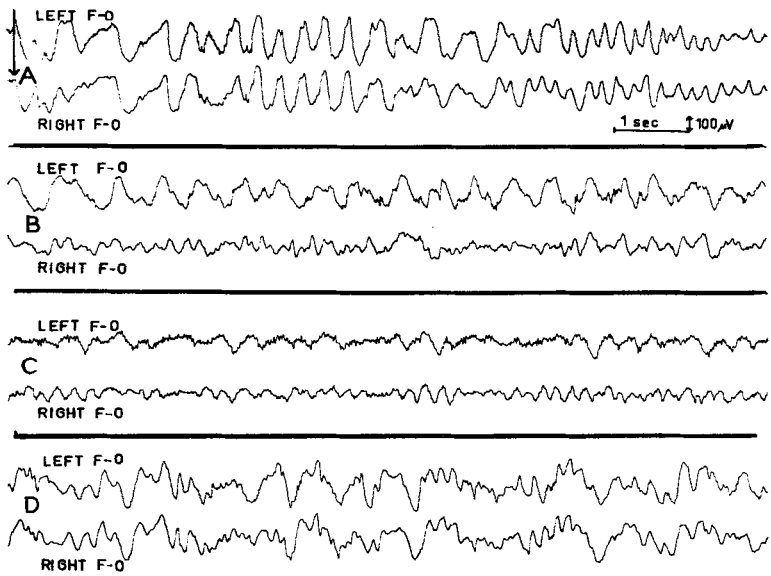

Fig. 1. *EEG effects induced by blood deprivation from the left hemisphere (urethane anesthesia).* A. Symmetric record with alerting effect obtained at the start of injection (see arrow) of Ringer's solution via the left internal carotid artery. B. Delta activity over the left hemisphere after blood deprivation for 80 sec. C. Persisting left-sided EEG changes 2 min after injection during i.v. administration of trypan blue. D. Symmetrical EEG pattern obtained at the end of the experiment (about 20 min). (F-O: bipolar fronto-occipital leads).

irrigation of the cerebral vessels the brain was removed. No staining of either hemisphere was observed.

Comment. This type of experiment demonstrates how unilateral ischemia affects the EEG without simultaneous influence on the blood-brain barrier to acid dyes. In addition to such analysis of ischemia effects *per se* it is possible to study how one hemisphere, previously subjected to ischemia of varying duration, reacts to certain intravenously given agents, *e. g.* analeptic drugs, in comparison with the other hemisphere.

2. *Effects induced by the organic acid Urokon®*

A rabbit weighing 1.8 kg, anesthetized with urethane, showed a symmetric EEG (Fig. 2 A) before application of Urokon (sodium acetrizoate). Via a carotid catheter 14 % Urokon (6 ml) was perfused through the left hemisphere for 45 sec. The EEG recording, starting 4 min after this injection, showed essentially the same symmetric pattern as before (Fig. 2 B). Ten minutes after the carotid injection the general circulation was loaded with Urokon by i.v. injection of 12 ml 40 % solution. A few minutes later the EEG showed a marked asymmetry with dominance of slow waves on the left side, especially after arousal stimulations. About 10 min after the i.v. injection frequent spikes with

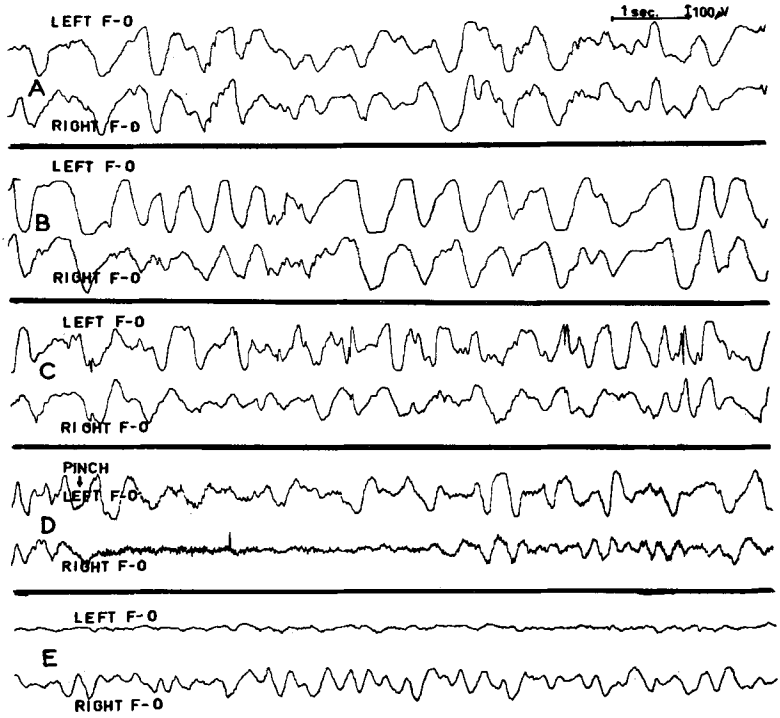

Fig. 2. *EEG changes after unilateral application of Urokon® (by left-sided carotid injection) and after subsequent intravenous loading with Urokon.* A. Control record (urethane anesthesia). B. Four minutes after left-sided carotid injection of 14 % Urokon (6 ml) for 45 sec. Essentially the same symmetric pattern as in A. C. Spike potentials on the left side starting about 10 min after i.v. administration of 12 ml 40 % Urokon. D. EEG recorded 2 hours later showing symmetric resting EEG but asymmetric arousal effects. E. Flat line on the left side about 15 min after the second i.v. injection of Urokon (40 %, 15 ml). (F-O: bipolar fronto-occipital leads).

high amplitudes were observed on the same side (Fig. 2 C). The abnormalities vanished within an hour but alerting stimuli still evoked transient asymmetric response (Fig. 2 D). A second i.v. injection of Urokon (15 ml, 40 %) was performed about two hours after the first. About 15 min later a marked depression of the amplitudes, practically down to flat lines, occurred on the left side, while no significant influence could be seen on the right side (Fig. 2 E). As intravital dye indicator 40 ml of trypan blue (0.5 %) was given i.v. in two portions, before and after the second Urokon administration. The rinsed brain showed a moderate degree of staining by trypan blue limited to the left hemisphere.

Comment. From earlier investigations the X-ray contrast medium Urokon (sodium acetrizoate) is known to cause barrier damage (BROMAN and OLSSON 1948 and 1956, BLOOR *et al.* 1951 a). When injected via the carotid artery in the concentration and with the technique used here this damage could be

expected to be of a low degree (STEINWALL 1958) and primarily affect the structures responsible for the barrier function. This supposition is in agreement with the observation that the EEG in this phase of the experiment did not show any significant changes. Afterwards when a large amount of Urokon was administered i.v. this polar compound could invade the hemisphere with the defective barrier and cause marked influence of the EEG on this side. Thus Urokon in this experiment played a double role as local barrier damaging agent and as indicator of the barrier defect. In either or both of these roles Urokon may be replaced by others of a large group of organic anions including many dyes (STEINWALL 1961).

Analogous studies on organic cation compounds are in progress at our laboratory with the purpose of supplementing the information on selective barrier inhibition (STEINWALL in press) as well as developing clinical methods for testing damage to the blood brain barrier (FLODMARK 1958, 1962).

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