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Metabolism of the Perfused Cat Brain During Electroanaesthesia

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MAGNES, J., C. ALLWEIS, S. HOCHERMAN, R. ASHKENAZI AND E. TAL. *Metabolism of the perfused cat brain during electroanesthesia.* PHARMAC. BIOCHEM. BEHAV. 1(5) 515–521, 1973.–The metabolism of the perfused cat brain was studied during the unresponsive state produced by electroanesthesia and was compared to control brain perfusion experiments. With external electrodes placed in the temporal muscles the rate of CO_2 production and oxygen consumption increased during the passage of current whereas the relative specific activity of CO_2 produced by the brain in the presence of U-^{14}C glucose was decreased. Lactate production by the brain increased. When smaller currents were passed bilaterally through electrodes placed in the deep layers of the superior colliculi, a similar unresponsive state resulted and the metabolic effects noted above were even greater. It is inferred that the passage of current in both cases produced widespread augmentation of neural activity and that the accompanying loss of consciousness and lack of responsiveness is therefore not due to a direct depressant anaesthetizing action as is the case with most chemical anesthetics. Possible mechanisms underlying this phenomenon are discussed.

Electroanesthesia Brain perfusion Metabolism

WHEN electrodes are placed upon the head of an animal and electric current is passed through the brain, under certain well-defined conditions, there may result a total lack of response to sensory stimuli. This state is usually called electroanaesthesia (EA) or electronarcosis [9]. Very little is known regarding the basic mechanisms underlying this unresponsive state induced by the passage of electric current. In a previous paper from this laboratory, Hocherman [8] showed that there is a bilateral region in the brain stem which is most sensitive to electroanaesthetic currents, and that the current required to produce a state of areflexia when passed through this area is from 1/10th to 1/100th that required when extracranial electrodes are used. The area under discussion is bounded by stereotaxic planes: A: 3.5–5.0; L: 1.5–3.5; H: 0.0–(+)3.0. It includes some of the mesencephalic reticular formation in the deep layers of the superior colliculi (SC) and it extends anteriorly into the region of the nucleus of the posterior commissure.

In view of our interest in the influence of different functional states on cerebral metabolism and circulation [10], a series of brain perfusion experiments was carried out to study the effects of passing electric current through the brain on metabolism and function in comparison to control brain perfusion experiments.

METHOD AND PROCEDURE

Isolation of Cerebral Circulation and Perfusion of the Brain

Isolation of the cerebral circulation was performed according to Geiger and Magnes [4] in cats of 2–3 kg body weight, anaesthetized with pentobarbitone. The principle of the isolation is that all venous outlets from the extracranial tissues are ligated so that the arterial blood can pass only through the brain. The natural venous outlets from the brain including the spinal venous sinuses are occluded as well and the cerebral venous blood is collected through screw cannulae inserted into the bone over the occipital sinuses near the base of the skull. It has been shown that with this method, negligible admixture of extracranial with cerebral blood takes place [3,4].

The brain was perfused at a constant flow rate [1] with simplified blood consisting of well-washed bovine red blood cells suspended in a Krebs-Ringer solution containing 7% bovine albumin (Fraction V) and 0.1% (w/v) glucose. In some experiments polyvinyl pyrrolidone (PVP) was used as the plasma expander in place of albumin.

The simplified blood was saturated at 37°C with 4% CO_2 in O_2 and the pH was adjusted to 7.4 by adding small amounts of acid or alkali as required. The pCO_2 was in the range of 26–30 mm Hg, the O_2 content 17–18% (v/v) and the haematocrit 36–38%. Before introducing the blood into the perfusion apparatus it was filtered through a Dacron wool filter [15]. Another Dacron wool filter was present in the perfusion circuit.

II. Data Collected

1. Functional. a. Continuous Recordings: (1) brain perfusion pressure; (2) respiration rate; (3) peripheral arterial blood pressure (femoral artery); (4) EKG; (5) ECOG from

screw electrodes in contact with the dura; (6) blood flow rate. *b. Periodic observations (5 min intervals):* corneal reflex response; pinna reflex response; light reflex response; visual following of a moving object; evoked response to sound (ECOG record).

2. Metabolic. Cerebral A-V O_2 differences (photometrically); cerebral V-A CO_2 differences (Van Slyke's method) periodically; cerebral A-V glucose differences in serum [7] periodically; cerebral V-A lactate differences in serum [16] periodically; cortical glucose content at the end of experiment; cortical lactate content at the end of experiment.

3. Calculations. Cerebral O_2 uptake – ml/100 g brain/min – ($\dot{V}_{O_2}$); cerebral CO_2 production – ml/100 g brain/min – ($\dot{V}_{CO_2}$); cerebral lactate production – ml/100 g brain/min – ($\dot{Q}_{lactate}$); relative specific activity of CO_2 produced by the brain from U-C^{14} Glucose – (RSA) (1).

4. Explanations to 1, 2 and 3. The reflexes tested (1b) were recorded on a scale 0–4. The evoked response to a click was periodically registered in the ECOG in the control experiments and in the electroanaesthesia experiments before and after application of current. During application of current ECOG could not be recorded because of electrical artefacts in the records caused by the anaesthetizing current.

We have not calculated respiratory quotients for the brain from our data because although the CO_2 determinations which are performed individually by the Van Slyke method are accurate, the oxygen uptake data as recorded continuously by a photoelectric oximeter is derived with the aid of a calibration curve. Owing to the unavoidable variation in the characteristics of the blood which is prepared for each experiment from a different animal this curve may shift slightly from experiment to experiment. Hence although the oximeter provides a reliable and sensitive indicator to changes in the rate of oxygen uptake during the course of a particular experiment, we cannot consider the absolute values obtained with it in a given experiment as sufficiently accurate for the calculation of a reliable R.Q. since the highest possible accuracy is necessary for that purpose.

III. Characteristics of Electroanaesthetic Currents

Electric current was applied either through external temporal electrodes or through stereotaxically positioned electrodes in the brainstem. With the external electrodes, positive rectangular current pulses, biased by a 4 mA D.C. positive current was used. The frequency of stimulation was 100/sec and the pulse duration 3 msec. The external electrodes were a pair of sharpened graphite rods 1.5 cm long and 2 mm in dia. and they were inserted into the temporal muscles [8]. With extracranial current application, unresponsiveness was achieved with peak currents of 20–40 mA.

Application of current to the brainstem was through 3 stainless steel insulated electrodes 0.5 mm in dia. with bared tips arranged in a row. The electrodes were stereotaxically positioned in the brain at the level of the superior colliculi (SC) and parallel to the frontal axis of the brain, at frontal plane A 4.0–4.5 to reach the horizontal plane H +1 to +2. The central electrode was in the midline and was maintained at zero potential. The two lateral electrodes were located 2.5 mm lateral on either side of the midline. The SC electrodes were both connected to the positive pulse – out terminal of the constant current pulse generator. With the SC electrodes we used unbiased rectangular pulses, 3 msec in duration at frequency of 100/sec. Areflexia was achieved with peak currents of 0.2–0.8 mA.

IV. Procedure

Three series of experiments were carried out.

1. The first was a preliminary exploratory series in which PVP was used as the plasma expander and stimulation with external temporal electrodes was carried out over a relatively long period. This series consisted of 3 experiments with electroanaesthesia and 3 control experiments in which no changes were carried out. In the experiments with EA, after perfusion had proceeded for about 30 min, a continuous square wave pulse-train was passed for about 1 hr between electrodes placed in the temporal muscles. Recovery was followed for 24 min.

2. In the second series albumin was used and there were two EA experiments and 2 control experiments. Stimulation with external temporal electrodes was carried out for a 15 min period beginning 60 min after the start of perfusion. The period of stimulation was shortened in order to allow more time to follow recovery. The first sample of blood during stimulation was taken 5 min after the start of stimulation in order to increase the resolution of the effects of stimulation in time.

3. The third series consisted of 3 EA experiments and 2 control experiments. Albumin was used as the plasma expander and weak stimulation was applied through SC electrodes for 15 min beginning 65 min after the start of perfusion.

RESULTS

In all the experiments breathing was normal during the application of current. Current was gradually increased until the pinna, corneal and light reflex scores were reduced to zero, by which time, visual tracking ability (when present) had also disappeared. The responses reappeared within 5 min after the cessation of current.

Brain perfusion pressure (which is an index of cerebral vascular resistance) rose sharply with the application of current and then came down immediately. It usually levelled off 5–15 mm Hg below the level recorded before EA was induced [11].

(a) PVP – Temporal Electrodes: Fig. 1.

$\dot{V}_{CO_2}$ decreased slowly during the control perfusions. This seems mainly to be due to the PVP since with albumin this does not usually occur. Within a few minutes after the onset of stimulation, $\dot{V}_{CO_2}$ increased by 30% on average and remained high till the end of stimulation. Subsequently it fell. The RSA of CO_2 produced by the brain had not reached an equilibrium value even at 120 min of perfusion while in the controls it had leveled off by about 80 min.

The rate of conversion of blood glucose to $C^{14}O_2$ increased by 80% in two experiments and by 40% in the third. The controls showed only slight increases if any over the corresponding period. The rate of oxygen consumption was significantly increased in all experiments compared with controls. Controls liberated little lactic acid. There was no significant difference during stimulation.

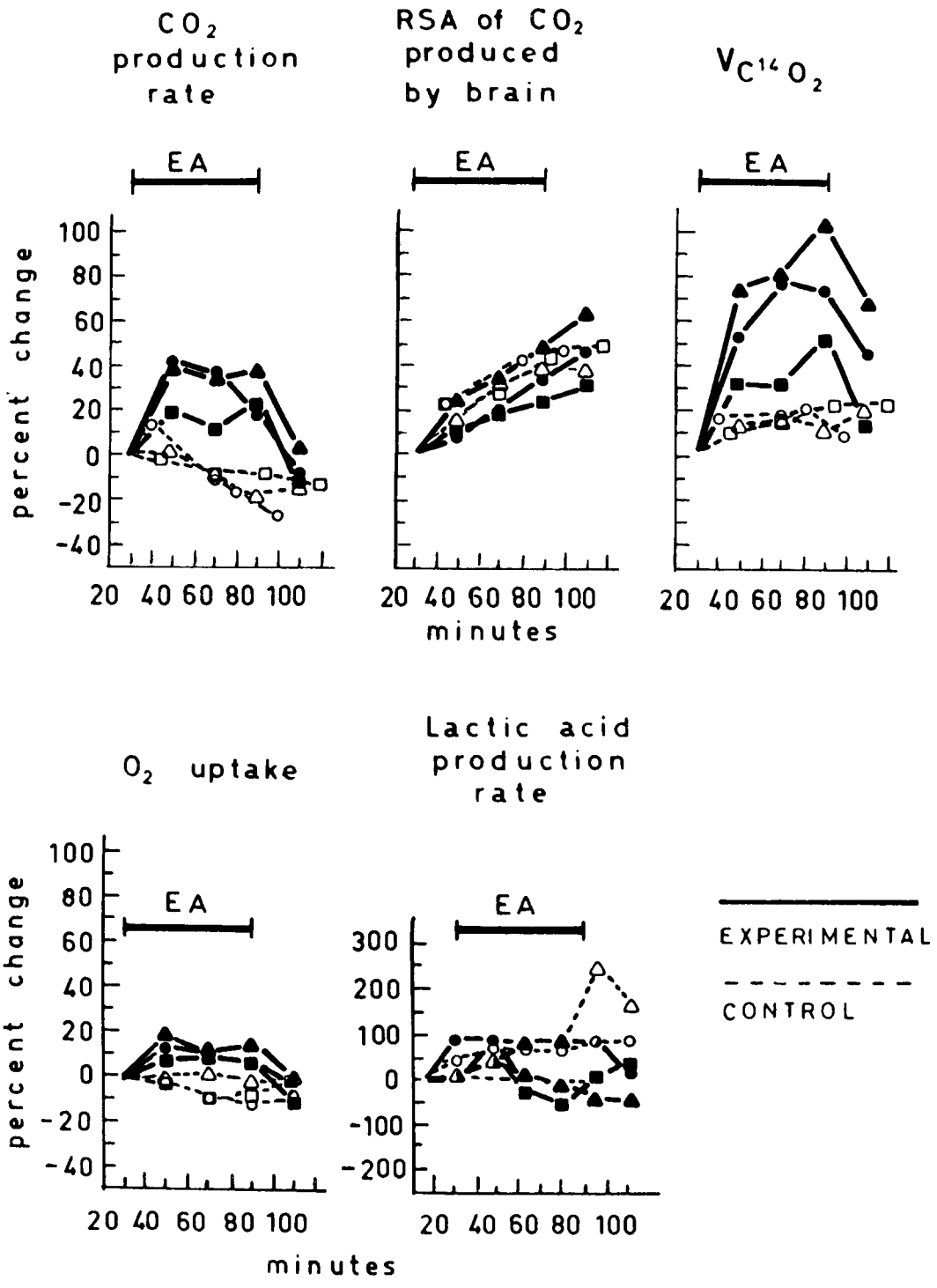

FIG. 1. The effect of stimulation of the brain with a continuous train of square wave pulses on five metabolic variables. The stimulation period is indicated on the graphs (EA). The simplified blood with which the brain was perfused contained PVP. The current passed between the extracranial electrodes in these experiments was 15–50 mA. The control experiments were identical except that the animals were not electrically anaesthetized.

(b) Albumin – Temporal Electrodes: Fig. 2a

$\dot{V}CO_2$ increased by 60% during EA which is double the increase obtained with PVP. The onset of this effect was abrupt. The RSA of the CO_2 fell sharply by about 20%, but the fall was not sustained. Recovery was complete in one experiment but slower in the other. The rate of production of $C^{14}O_2$ showed a marked rise. The rate of oxygen consumption showed a rise of about 30% followed by a

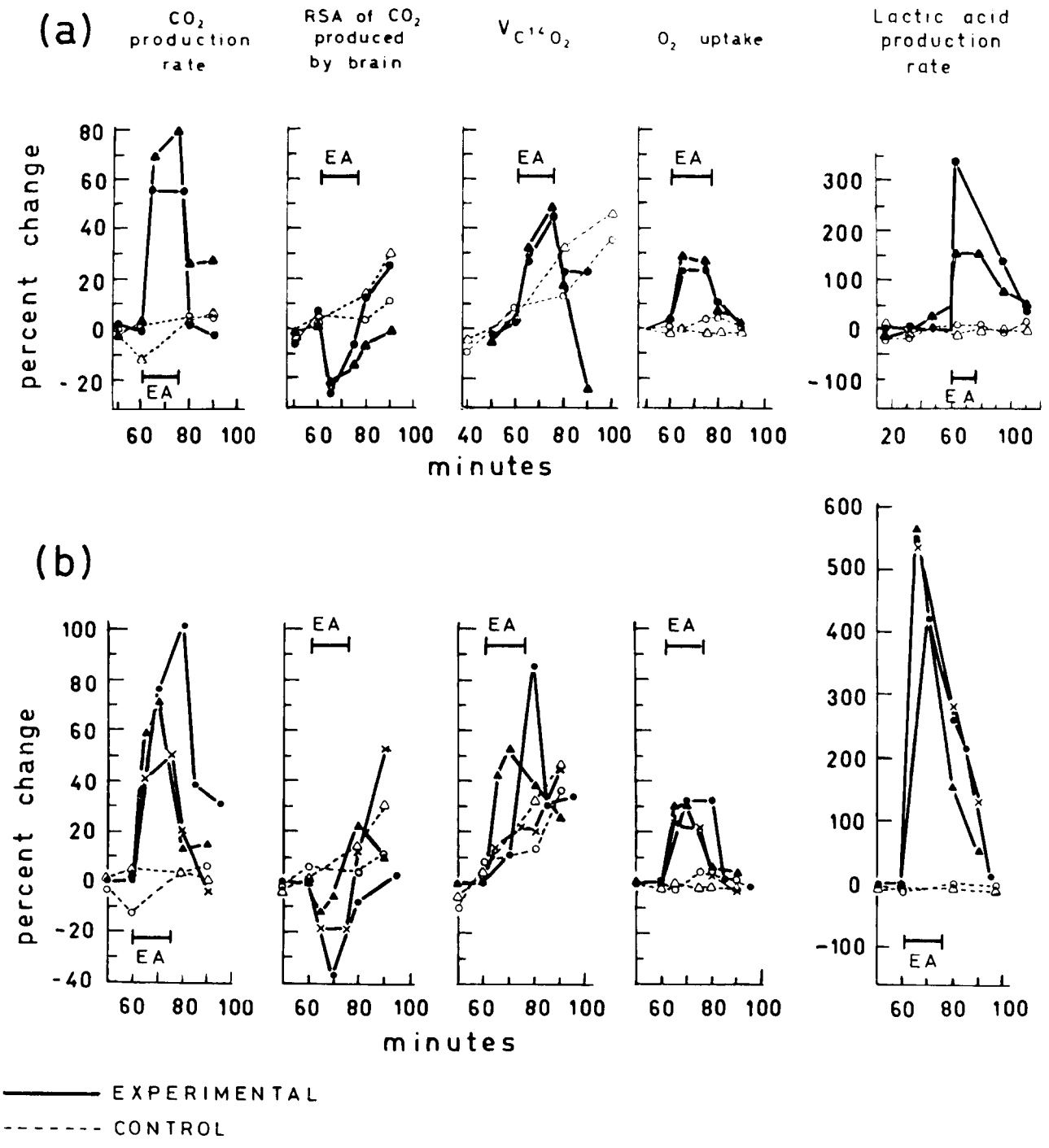

FIG. 2a. The effect of stimulation of the brain with a continuous train of square wave pulses on five metabolic variables. The stimulation period is indicated on the graphs (EA). The simplified blood contained 7% of bovine albumin. The current passed between the extracranial electrodes in these experiments was 2–12 mA.

FIG. 2b. The effect of stimulation of the brain stem with a continuous train of square wave pulses on five metabolic variables. The stimulation period is indicated on the graphs (EA). The simplified blood contained 7% bovine albumin. The stimulating eelctrodes were stereotaxically directed to the superior colliculi (see Method). The current passed was 0.2–1.1 mA.

The control experiments were identical except that the animals were not electrically anaesthetized.

rapid fall when stimulation was discontinued. $\dot{Q}_{lactate}$ rose abruptly by 100% and 35%. It fell rapidly with the cessation of stimulation.

Figure 3 relates the amount of electroanaesthetic current applied, the percentage increase in the rate of production of CO_2 and the physiological state of the animal in the experiments of Series b.

At the lowest current used, 800 μA, there was no discernible change in the state of the animal and no significant change in metabolic rate. Whilst we do not have enough data to be certain on this point, it appears that as the current increases up to about 8 mA the CO_2 production rate increases rapidly. With further increase (the 30 mA experiment was done by mistake!) little additional increase in the CO_2 production rate is seen. The dotted line on the graph is a rough estimate only.

In all experiments in which the CO_2 production rate was increased by the electrical current by more than about 30% the animal was found to be unresponsive.

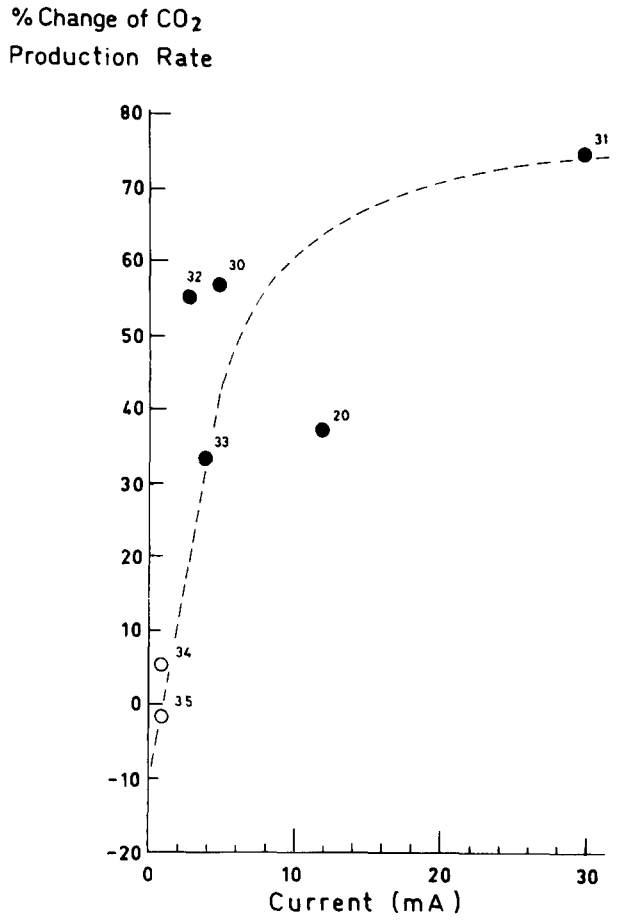

FIG. 3. The relationship between electric current, percentage increase in rate of production of CO_2 and physiological state (as determined by reflex activity) in the experiments with external temporal electrodes and albumin in the blood. Points marked ○ represent animals in which the functional state of the brain did not appear to be altered by the current. The numbers alongside the points are experiment numbers.

(c) Albumin – Superior Colliculi Electrodes: Fig. 2b

Within a few minutes after the onset of stimulation the rate of production of CO_2 by the brain rose by about 70% (100% in one experiment). The fall was rapid after stimulation ended.

The RSA of the CO_2 produced by the brain fell in all experiments. Recovery was rapid. The rate of production of $C^{14}O_2$ showed a marked rise in two experiments. The rate of oxygen consumption in all experiments rose abruptly by about 30% and fell rapidly when stimulation ceased. $\dot{Q}_{lactate}$ rose by 500% immediately after stimulation began but fell during and after stimulation.

A summary of the main results of the three series of experiments is presented in Table I. Glucose uptake is not included because it was found to be extremely variable and did not follow any consistent pattern.

DISCUSSION

Our preliminary investigation using PVP showed the feasibility of the experiments we intended to perform, but in the light of subsequent work using albumin it appears that PVP, in some unknown way, limits the extent to which the metabolic rate of the brain changes under the influence of electric current. The discussion will therefore be concerned mainly with the results obtained with bovine albumin which is probably a more physiological constituent of blood than PVP.

Our findings with respect to the loss of spontaneous movements, loss of reflexes and loss of reactivity to painful stimuli are in agreement with reports by other workers who have studied the effects of passing electric currents through the brain [13]. However, our investigations of the metabolism of the brain under these conditions reveal an unexpected pattern which is quite similar to that produced by electroshock or the administration of metrazol in convulsant doses.

In earlier studies it was found [2] that though CO_2 production rate is increased during metrazol convulsions and during electroshock convulsions, the RSA of the CO_2 produced by the brain decreases. When however pentobarbital was given to the perfused brain in sufficient amount to just cause the disappearance of reflexes the CO_2 production rate decreased by about 50% but the RSA values did not change [10]. This is clearly brought out in Fig. 4 which includes some of the results of this investigation and data from earlier studies [2,10]. The main point to be made therefore is that the present results with EA are similar to those obtained with convulsions and quite different from those obtained with a chemical anaesthetic.

Stimulation through SC electrodes with low currents (about 0.5 mA) which were adequate for the production of the unresponsive state also brought about abrupt dramatic increases in CO_2 production and oxygen consumption which were as large or larger than those produced when stronger currents (up to 30 mA) were passed between extracranial electrodes. Since this implies at least an equal degree of augmentation of neural activity in both cases, it suggests that there exists a neural system capable of propagating activity initiated in the region of the electrode tips to large masses of neural tissue at varying distances from it. In fact, neurons from the nucleus of the posterior commissure fire not only downwards into the spinal cord but upwards to the subthalamus and to the interlaminar nuclei of the

TABLE 1

SUMMARY OF RESULTS

	NUMBER OF EXPERIMENTS	NUMBER OF CONTROLS	mA	% CHANGE (↑ INCREASE ↓ DECREASE)				
				$\dot{V}_{CO_2}$	RSA	$\dot{V}_{C^{14}O_2}$	$\dot{V}_{O_2}$	$\dot{V}_{Lactate}$
P.V.P.	3	3	15 - 50	30 ↑	0	45 ↑	15 ↑	0
Albumin: temporal electrodes	2	2	2 - 30	60 ↑	30 ↓	20 ↑	20 ↑	200 ↑
Albumin: Sup. Coll. electrodes	3	2	0.2 - 1.1	70 ↑	20 ↓	30 ↑	30 ↑	500 ↑

A summary of the metabolic changes, produced by the passage of electric current through the perfused cat brain in comparison to control perfusion experiments. (The percentages are average values.)

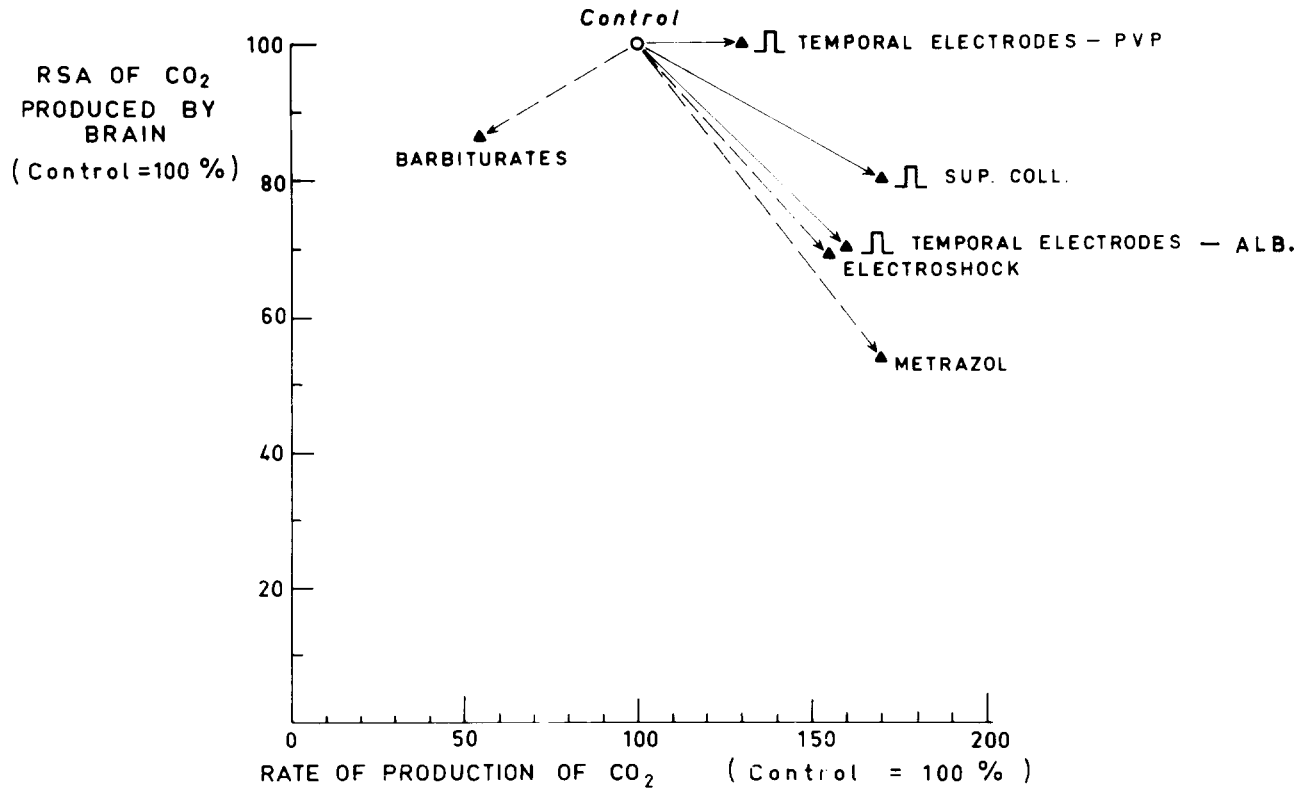

FIG. 4. Changes in the rate of production and RSA of CO_2 produced by the perfused cat brain caused by barbiturates, metrazol, convulsive electroshock, and the passage of current through the brain. It will be noticed that the changes produced by the passage of current through either temporal electrodes or electrodes in the superior colliculi are much closer to those produced by electroshock than to those produced by barbiturates. The response in the presence of PVP is very limited.

thalamus which have massive projections to the cortex. This could result in massive activation of cortical neurons and a resulting large increase in cerebral metabolic rate (CMR).

In 1955 Geiger and Sigg [5] described constant-pressure brain perfusion experiments in which they electrically stimulated various parts of the brain stem. Stimulation of the posterior hypothalamus and of various points in the midbrain recticular formation caused an increase in CMR which was not sustained for the duration of stimulation. Since the reactivity of the animals in these experiments was not reported it is not possible to know whether the metabolic phenomena they described are necessarily linked with a loss of reactivity or may be dissociated from it.

The rates of consumption of oxygen and production of CO_2 by neural tissue are generally considered to be the sum of basal resting rates and an increment whose value is proportional to the activity of the neural elements in the tissue [14].

It is clear that the lack of responsiveness in our experiments is not associated with decreased neural activity and a fall in metabolic rate as is the case in pharmacological anaesthesia. Although direct measurements of the average rate of firing of neurons during the passage of current were not made, our findings suggest that it is greatly increased during the unresponsive state induced by the passage of electrical currents through the brain. It is possible that the hyperactivity disrupts the normal intricate spatial and temporal patterns of impulse initiation and transmission which is essential both for the conditon we recognize and term consciousness, and also for the objectively demonstrable responsiveness to various stimuli. Alternatively, the lack of responsiveness during stimulation may be due to the activation of inhibitory pathways that pass through this area of the brain as suggested by Hocherman [8]. It is also possible that both mechanisms interact to produce the unresponsive state. The fact that stimulation of the SC area causes disappearance of reflexes while the ECOG continues to be desynchronized [8] might be explained by assuming that the SC electrodes activate the ascending reticular activating system and simultaneously block by polarization the classical ascending sensory pathways. It is interesting that Hocherman who recorded the ECOG in a few of his experiments, using a track and hold circuit, found it to be desynchronized during EA and that the amplitude of the activity was slightly lower during passage of current than without it [8].

Whichever hypothesis is correct, it is quite clear that the state of the brain during the passage of current is quite different from the state of the brain during pharmacologically induced anaesthesia. In fact the metabolic behavior of the brain is very similar to that found by us during convulsions produced by electroshock or metrazol [2]. Thus the names electroanaesthesia or electronarcosis are misleading. In view of the interest of anaesthesiologists and surgeons in exploiting electrical currents as an anaesthetic agent for carrying out surgical procedures in animals and man [6, 12, 13] it is felt that further research in this area is urgently required.

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