Research report

Haemodynamic correlates of penumbral depolarization following focal cerebral ischaemia

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Abstract

Transient ischaemic depolarizations (IDs) are thought to play a key role in the pathogenesis of focal cerebral ischaemia. Most transient IDs are akin to spreading depression (SD), although a negative DC shift is not observed in half the cases. The other IDs may represent transient anoxic depolarizations. Using cortical DC and blood flow recordings, following middle cerebral artery occlusion in rats, we show here that: (i) these later depolarizations do indeed represent transient anoxic depolarizations; (ii) SD-like IDs, DC and haemodynamic parameters are similar to those of SDs when blood flow remains close to baseline and; (iii) when blood flow decreases, the hyperaemia associated with SD-like IDs is largely reduced and there is an increasing proportion of cortical sites which fail to display a DC shift. These data demonstrate the coexistence of two mechanisms of IDs, and yield new information as to the flow-dependence of DC and haemodynamic correlates of SD-like IDs, the pathophysiological significance of which remains to be determined.

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1. Introduction

In the first hours following middle cerebral artery occlusion (MCAo), the transient ischaemic depolarizations (IDs) that occur intermittently in the penumbra are thought to be involved in the final infarction. We have previously demonstrated [18] that different types of IDs coexist in the penumbra. While their characteristics (time of occurrence, amplitude, duration and propagation) have been described in detail, their relationships with the underlying haemodynamic status remain unclear.

About 10% of IDs, the duration of which is more than 7 min (long IDs), occur essentially in the first hour following ischaemia and have characteristics markedly different from those of spreading depression (SD) and therefore cannot be assimilated to the phenomenon of SD. We hypothesize that such IDs are transient anoxic depolarizations (ADs) and are thus associated with a reversible decrease in cerebral blood flow.

The second type of event, with a duration of less than 7 min (short IDs), are akin to SD, a phenomenon originally described by Leão [15] as a reversible and transient negative shift of the direct current (DC) which spreads over the cortex at a rate of 2–5 mm/min. One hypothesis, that would explain the deleterious role of SD-like IDs, is that repolarization requires extra energy which cannot be supplied due to the limited haemodynamic reserve in the penumbral tissue. While SD is coupled to a compensatory rise in CBF and does not cause damage in an intact animal, the presence or not of a hyperaemia associated with SD-like IDs remains to be clarified [1,10].

In addition, we have previously showed that, with ongoing ischaemia, some cortical sites intermittently fail to display a DC shift and are entirely replaced by suppressed electrocorticographic activity, while an ID wave is observed on the other electrodes [18]. We have referred to these events as ECoG silences. The relationship between

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the proportion of ECoG silences and the residual blood flow is also unknown.

Accordingly, the aim of our present study was to clarify the haemodynamic correlates of the various alterations in ionic homeostasis that follow MCAo. This characterisation should enable the determination as to whether long IDs represent AD and to clarify the relationship between the residual blood flow and the hyperaemia associated with either IDs or the proportion of ECoG silences.

2. Materials and methods

Adult male Sprague–Dawley rats weighing 308±30 g were assigned to two experimental groups: a MCAo or ‘ischaemia’ group (n=16), and an electrically-induced SD or ‘control’ group (n=4). Experimental procedures were performed according to the appropriate European directives and French national legislation.

2.1. General preparation

Anaesthesia with halothane (4%) was induced and maintained (1.5–2%) during surgery in a mixture of O₂:N₂O (30:70) with the animal breathing spontaneously (n=11, ischaemia group) or intubated and artificially ventilated (n=5 for ischaemia group, n=4 for control group). A catheter was inserted into the femoral artery for arterial pressure monitoring and to obtain samples for gas analysis. Rectal temperature was kept close to 37.5°C with a feed-back controlled heating pad.

2.2. Electrophysiological recordings

The animal’s head was fixed in a stereotaxic frame and the skull was exposed. A right frontoparietal craniotomy (8 mm×4 mm) was performed and the dura mater was left intact, laterally between the level of the lambda and the bregma, so as to insert (2 mm apart) four glass microelectrodes (E1 to E4; tip diameter, 8 µm) filled with physiological saline, 200 µm deep into the cortex, within the MCA territory (stereotaxic coordinates: all electrodes 3 mm lateral to the sagittal suture, E1 1 mm posterior to bregma, E4 7 mm posterior to the bregma, E2 and E3 in between). Ag/AgCl wires were inserted into the microelectrodes and connected to an amplifier. An Ag/AgCl reference disk electrode was inserted under the skin of the neck.

2.3. Cerebral blood flow recordings

Cerebral blood flow (CBF) was measured continuously by laser doppler flowmetry (LDF). One or two LDF probes were placed over the dura mater close to the electrodes while carefully avoiding all visible vessels. Saline was circulated over the meninges and the surface temperature was controlled by a microthermocouple and kept close to 37.5°C. The cortical potential and CBF were recorded for 20 min prior to MCAo or stimulation, and over the following 4 to 5 h. Signals were digitized using LABTECH software (Laboratory Technologies Corporation, UK). During the recordings, the concentration of halothane was reduced to 0.8–1%.

2.4. Middle cerebral artery occlusion

In the ischaemia group, permanent focal cerebral ischaemia was induced by a remote controlled method of intraluminal MCAo [20]. A cylinder of thermofusible adhesive (0.38×2 mm), attached to a nylon thread (diameter 0.22 mm), was advanced from the lumen of the external carotid artery (ECA) into the internal carotid artery, 5 mm beyond the surface of the skull. A catheter was placed through the portion of the thread remaining outside the ECA stump, inserted into the stump and secured. Subsequently, the MCAo was achieved by advancing the thread by a further 4 mm.

2.5. Electrically-induced spreading depression

In control animals, an additional right frontal craniotomy (diameter 2 mm) with the dura mater intact was performed 2 mm rostral to the bregma and 2 mm lateral to the sagittal suture. Two insulated Ag/AgCl wires, were placed 2 mm apart between the calvarium and the meningeal surface. Trains of stimuli (2 ms pulses, 8 mA, at 80 Hz for 10 s) were then delivered every hour for 5 h.

2.6. Data and statistical analyses

Recordings were analysed using DADISP (DSP Development, UK) and Statview (Abacus Concepts, USA). Blood flow was expressed as percentage of base line. Blood flow changes ( hyperaemia) were expressed in arbitrary units; 1 unit equals 1% of base line. Electrophysiological and CBF data were analysed by ANOVA. Each LDF recording was analysed in relation to the electrophysiological data arising from the closest electrode. Repeated-measures ANOVA followed by Student’s t-test and a Bonferroni correction were used to compare physiological parameters between the groups. Contingency tables were analysed by the χ² test. Values are given as mean±S.E.M. and P<0.05 was accepted as significant.

3. Results

3.1. Physiological variables

There were no significant differences in physiological parameters between the ischaemia and control groups. Within the ischaemia group, a significant difference between spontaneously breathing and artificially ventilated
animals was observed only for PaCO\textsubscript{2} (46.4±1.4 mmHg and 38.8±1.4 mmHg, respectively; \(P=0.005\)) and, as would be expected, for pH\textsubscript{a} (7.34±0.01 and 7.39±0.01, respectively; \(P=0.04\)). The other systemic parameters were: PaO\textsubscript{2}=130±5 mmHg; MAP=92±2 mmHg.

3.2. Ischaemia group

3.2.1. CBF and DC/ECoG recordings

Immediately following MCAo, the residual CBF ranged from 100 to 9.6\% of the base line values. A terminal AD occurred in only one animal with a CBF of 15\% of control values. In all other animals, IDs affecting sequentially all or some of the electrodes (98 series of events, each observed at four locations, corresponding to 392 ischaemic events) were noted at irregular intervals, were reversible and were of variable duration. Based on the criteria previously validated in our model [18], two populations of IDs were separated according to their duration: long IDs (4.3\% of events; duration: 23.4±3.6 min; amplitude: 24.2±2.8 mV) and SD-like depolarizations (55.4\% of events; duration: 2.1±0.1 min; amplitude: 16.6±0.5 mV). The remaining events (40.3\%) were ECoG silences. The total number of recorded events decreased progressively with the post-occlusion time (Fig. 1). To test the hypothesis that ECoG silences could be due to refractoriness, we calculated the average lag time between an ECoG silence and the previous event. An ECoG silence occurred on average 22±24 min after the preceding event wave.

3.2.2. CBF changes associated with long IDs

Most long IDs (70.6\% of long IDs) were associated with a decrease in CBF to 18.1±5.7\% of base line values and, at the time of repolarization, with a recovery of CBF towards pre-ischaemic values (73.6±14.7\% of base line values) (Fig. 2A).

These long IDs were recorded as the first electrophysiological event and occurred on all of the four electrodes. No long IDs were observed twice in the same animal. The duration of the remaining long IDs was 9.8±0.9 min, a value slightly above our threshold and these events were therefore considered SD-like depolarizations (duration of SD-like IDs to include these later events: 2.3±0.1 min; amplitude: 16.6±0.5 mV).

3.2.3. CBF changes associated with SD-like IDs and ECoG silences

SD-like depolarizations and ECoG silences occurred...
Fig. 2. Examples of CBF changes associated with electrophysiological ischaemic events in four different animals. The LDF probe and electrode are approximately 1~2 mm apart. (A) Transient decrease in CBF associated with a long ID; (B) Hyperaemia associated with SD-like ID at normal CBF values; (C) Hyperaemia associated with a SD-like ID and ECoG silence (second event) at a residual flow value of 80% of base line; (D) Insignificant variations in CBF associated with two SD-like depolarizations at a residual flow value of 60% of base line.

when the residual CBF was greater than 25.5% of pre-ischaemic values and were associated with either no change or a variable increase in CBF (Fig. 2B, C, D). The data were analysed as a function of the residual CBF, binned by intervals of 10%; only the electrophysiological data arising from the electrode closest to each laser-doppler probe was taken into consideration. It must be noted that we cover a wide range of residual CBF because the residual CBF varies from animal to animal. In each rat, residual flow was similar at the two LDF probe locations. The average amplitude of the hyperaemia associated with SD-like depolarizations was a function of the residual CBF: the greater the residual CBF, the greater the hyperaemia (Fig. 3). The percentage of ECoG silences relative to the total number of events was significantly related to the residual CBF (Fig. 4); it was greater at low flow rates than at normal blood flow values. The duration of IDs (taken overall) was significantly dependent on residual flow ($P<0.012$) though this was not the case for SD-like IDs taken alone ($P>0.91$). The amplitude of SD-like IDs was not dependent on residual flow ($P>0.11$).

3.3. Control group

In the control group, a deflection of the DC potential (duration: 1.08±0.03 min; amplitude: 22.4±0.5 mV), was observed nearly universally after stimulation (99.4%). SDs were associated with a hyperaemia which was also dependent on the pre-event blood flow (which spontaneously ranged from 60 to 120% of base line in this group): in this case, the greater the pre-event flow, the lesser the hyperaemia (Fig. 3). The amplitude and duration of SDs were not dependent on the pre-event flow.

3.4. Comparison between ischaemic and control groups

Finally, the parameters cited above (amplitude of hyperaemia, amplitude and duration of SD-like IDs and SDs) were analysed by a two-way ANOVA (pre-event CBF; group) when only the residual or pre-event CBF from 60 to 110%, due to missing values, was taken into consideration. With respect to the amplitude of the hyperaemia, there was an interaction between the group and the pre-event...
flow effects ($P<0.0001$) and the amplitude was significantly different between the two groups at flows ranging from 60 to 90% of base line (Fig. 3). With the electrophysiological data (amplitude and duration), there was no interaction between the group and the pre-event flow effects ($P>0.52$) but a significant group effect ($P<0.02$).

4. Discussion

The results of the present study demonstrate that: (i) while IDs are most often SD-like depolarization waves, they can also correspond to transient anoxic depolarizations; (ii) when blood flow remains normal after MCAo, SD-like IDs and their haemodynamic effects have characteristics identical to those of SDs; and (iii) when residual blood flow decreases, an increasing proportion of cortical sites fail to display a DC shift during an SD-like wave occurring on the others electrodes and these waves are associated with a reduced hyperaemia.

Several previous publications have highlighted the heterogeneity of IDs [5,9,16,17,19]. Based on the temporal profile of extracellular potassium increase [19], their duration [5] or their time of occurrence [5], some IDs have been postulated to correspond to an anoxic depolarization. These hypotheses were based on investigations in which blood flow was not measured. As in the present study, long IDs occurred shortly after the initial fall in blood flow and repolarization followed the recovery of blood flow, these long IDs can be assimilated to anoxic depolarizations. The initial decrease in CBF may be accompanied by an ischaemia-induced increase in the collateral circulation. While it is not known as to whether transient IDs occur in man following stroke, novel methodologies such as magnetic resonance imaging [7,13] and magnetoencephalography [2,3] are becoming increasingly available to reliably investigate this issue in patients. The distinction between the two types of IDs will be essential in these studies, since the pathophysiological significance and the potential pharmacology are different in each case.

The second type of IDs (short ones) have been shown to possess characteristics close to those of SDs [18]. In the present study, we have further defined the identity of IDs and SDs. When blood flow is close to normal following
MCAo, both the electrophysiological characteristics and the associated hyperaemia of IDs are similar to those of SDs. However, when blood flow is decreased, these characteristics no longer match those of SDs.

With respect to their electrophysiological characteristics, it would appear that ECoG silences are more frequent the lower the residual blood flow. The basis of this phenomenon is not known. ECoG silences occur locally during an SD-like wave (which can be seen as a depolarization on other electrodes): they are part of the wave but crucially lack the attributes of depolarization. Since the absolute refractory period following a spreading depression has been reported to be of 1 to 3 min, the mechanism of ECoG silences, which occurred on average 22 min after the preceding wave of event in our experimental conditions, cannot be explained by refractoriness. It has been shown from in vitro studies that, during SD, an intracellular calcium wave precedes that of the increased extracellular potassium [12]. Based on these facts, one might envisage that ECoG silences correspond to the effects of the calcium wave alone. Alternatively, these ECoG silences could reflect a SD-like wave occurring in close vicinity but not at the recording electrode, and therefore manifested as a deafferentation-induced silence. We speculate that the absence of depolarization is an adaptive mechanism which may spare intracellular ATP concentrations. Based on this hypothesis, the opening of K\textsuperscript{+}-ATP channels by a deficiency in ATP [11] would explain both the suppression of the potassium efflux and its dependence on blood flow, since the hyperpolarization due to the opening of ATP-dependent K\textsuperscript{+} channels would counteract the depolarization and since ATP concentrations are expected to decrease at lower rates of flow.

In our present study, we failed to find a significant relationship between residual blood flow and the duration of SD-like IDs. However, the results of a previous comparable study, as well as those of the present one when all IDs are pooled, show that decreased blood flow is markedly correlated with an increased duration of IDs [17]. This observation is the basis for a hypothetical mechanism for the deleterious role of IDs at low flow rates: repolarization is greatly compromised and becomes impossible around the ischaemic focus. Since long-lasting IDs occurred at markedly reduced flow levels in the above cited study, they may represent transient anoxic depolarizations, as in the present study, and therefore introduce a bias in the correlation between SD-like IDs duration and residual blood flow. Thus, we believe that the increased
duration of SD-like IDs has been largely overestimated in the literature.

Spreading depression has been nearly universally associated with a hyperaemia and our data confirm that this increase in blood flow is inversely proportional to the base line flow [14]. Under ischaemic conditions, our data confirm that, at low residual rates of flow, the hyperaemia becomes negligible or absent [1]. This finding is in agreement with the idea that the deleterious effects of SD-like IDs are due to a reduced haemodynamic reserve in the penumbra and, therefore, to a mismatch between energy requirements and availability. This hypothesis is challenged, however, by data which show that, in the absence of an ischaemic focus but with a reduced blood flow, SDs are not deleterious even when the total period of depolarization lasts up to 70 min [8]. It has been proposed that an even more reduced blood flow or the presence of an ischaemic focus is necessary for SDs to induce damage [8]. Another hypothesis can be invoked. We have here demonstrated that the hyperaemia is proportional to residual blood flow. When blood flow is in the physiological range or only slightly reduced, the hyperaemia is similar to that seen following SD. Since a wave of depolarization induces an increase in blood flow only in those tissues in which perfusion is not severely reduced, i.e. in the periphery of the ischaemic focus, a steal phenomenon could occur. Although we did not observe a decrease in blood flow during the SD-like IDs seen at low residual rates of flow, one might envisage that this phenomenon exists in the vicinity of the ischaemic focus, where a further minor decrease in flow may be sufficient to attain the threshold for membrane failure. In addition, this later hypothesis would explain the neuroprotective effects of the inhibition of neuronal nitric oxide synthase [6], a pharmacodynamic effect which is reported to block the hyperaemia associated with SDs [4] and would accordingly be expected to increase brain damage rather than to decrease it. In conclusion, two mechanisms of transient IDs coexist: one is a decrease in CBF followed by a recovery, which induces transient anoxic depolarization; the second is spreading depression. SD-like IDs propagate to all cortical recording sites no matter the level of blood flow. With a normal flow, SD-like ID electrophysiological and haemodynamic effects are identical to those of SDs. With reduced flow, the hyperaemia associated with SD-like IDs is less prominent and a large proportion of the waves fail to display the depolarization in the near entirety of the recording sites; the pathophysiological significance of this phenomenon remains to be determined. These findings should be useful for the interpretation of magnetic resonance images following cerebral ischaemia in both experimental and clinical studies. Finally, our current observations open new research perspectives towards the understanding of the mechanisms and roles of the alterations of the characteristics of SD secondary to a reduced blood flow.

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References


