ET-1 induces cortical spreading depression via activation of the ET<sub>A</sub> receptor/phospholipase C pathway in vivo

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ET-1 induces cortical spreading depression via activation of the ET<sub>A</sub> receptor/phospholipase C pathway in vivo. Am J Physiol Heart Circ Physiol 286: H1339–H1346, 2004. First published December 4, 2003; 10.1152/ajpheart.00227.2003.—Recently, it has been shown that brain topical superfusion of endothelin (ET)-1 at concentrations around 100 nM induces repetitive cortical spreading depressions (CSDs) in vivo. It has remained unclear whether this effect of ET-1 is related to a primary neuronal/astroglial effect, such as an increase in neuronal excitability or induction of interastroglial calcium waves, or a penumbral-like condition after vasoconstriction. In vitro, ET-1 regulates interastroglial communication via combined activation of ET<sub>A</sub> and ET<sub>B</sub> receptors, whereas it induces vasoconstriction via single activation of ET<sub>A</sub> receptors. We have determined the ET receptor profile and intracellular signaling pathway of ET-1-induced CSDs in vivo. In contrast to the ET<sub>B</sub> receptor antagonist BQ-788 and concentration dependently, the ET<sub>A</sub> receptor antagonist BQ-123 completely blocked the occurrence of ET-1-induced CSDs. The ET<sub>B</sub> receptor antagonist did not increase the efficacy of the ET<sub>A</sub> receptor antagonist. Direct stimulation of ET<sub>B</sub> receptors with the selective ET<sub>B</sub> agonist BQ-3020 did not trigger CSDs. The phospholipase C (PLC) antagonist U-73122 inhibited CSD occurrence in contrast to the protein kinase C inhibitor Gö-6983. Our findings indicate that ET-1 induces CSDs through ET<sub>A</sub> receptor and PLC activation. We conclude that the induction of interastroglial calcium waves is unlikely the primary cause of ET-1-induced CSDs. On the basis of the receptor profile, likely primary targets of ET-1 mediating CSD are either neurons or vascular smooth muscle cells.

endothelin-1; endothelin receptor antagonists; interastroglial calcium waves; gap junction communication; protein kinase C inhibition; arachidonic acid; cortical spreading depression

ENDOTHELIN (ET)-1 was initially believed to influence brain functions only indirectly through regulation of cerebral perfusion due to its vasoconstrictor activity. However, over the past few years, evidence has accumulated that ET-1 may fulfill a wider range of physiological actions within the central nervous system (CNS), which has also led to its recognition as a neuropeptide (for reviews, see Refs. 33 and 36). Beside neurons, astrocytes represent a major ET target. Several studies have shown that ET-1 affects numerous cell functions of cultured astrocytes such as control of ion channel activity (5), glutamate efflux (52) and uptake (41), glucose utilization (58), permeability of gap junction communications (3, 16), and calcium signaling (3, 64). In turn, astrocytes are a major source of ET-1 (12, 13) and seem to play an important role in the regulation of ET-1 levels within the CNS (14, 24, 25).

In contrast to the cerebrovascular system, in which ET<sub>A</sub> receptors are the major receptor subtype, astrocytes predominantly express the ET<sub>B</sub> receptor subtype (26, 38). However, at least in cultured astrocytes, there has also been evidence of ET<sub>A</sub> receptor mRNA expression (13) and an ET<sub>A</sub>/ET<sub>B</sub> hybrid receptor has been postulated (31). This unusual receptor pattern may be involved in certain actions of ET-1, such as increased glutamate efflux, evocation of calcium signaling, and controlling gap junction communication in cultured astrocytes (3, 52).

An interesting feature of astrocytes is their long-range signaling between themselves and neurons through intercellular gap junction-mediated calcium waves (18, 55). Such calcium waves can lead to depolarization of surrounding neurons and have been suggested to play a role in the initiation and propagation of cortical spreading depressions (CSDs) in vitro models (Ref. 45; for a review, see Ref. 42). ET-1 is known to evoke such intercellular calcium waves in cultured astrocytes (3, 63) and regulates their expansion by gap junction channel closure (3, 16). Interestingly, these effects of ET-1 on astrocytes are only inhibited significantly if both ET<sub>A</sub> and ET<sub>B</sub> receptors are simultaneously blocked (3). This is in contrast to several ET-1 effects on the vasculature or neurons that are inhibited by the application of either ET<sub>A</sub> or ET<sub>B</sub> receptor antagonists (48, 59, 66, 67). In a complex system such as the brain in vivo, it may be difficult to decide which cellular targets/functions mediate the complex effect of ET-1 on the neuronal/astroglial network. Studying the receptor profile/intercellular signaling cascade using receptor agonists and antagonists may help to limit the number of candidate cell types and functions for a given network effect. Here, we applied such an approach to analyze the recently discovered potent and reliable generation of CSDs by ET-1 in vivo (8).

MATERIALS AND METHODS

Animals

All animal experiments were approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz, und Technische Sicherheit Berlin (G 0346/98). Male Wistar rats (n = 101, 280–380 g) were anesthetized with 100 mg/kg thiopental sodium intraperitoneally (Trapanal, BYK Pharmaceuticals; Konstanz, Germany), tracheotomized, and artificially ventilated (Effenberger Rodent Respirator, Effenberger; Pfaffing/Attel, Germany). The left femoral artery and vein were cannulated, and a saline solution (1 ml/h) was continuously infused. Body temperature was maintained at 38.0 ± 0.5°C using a heating pad. Systemic arterial pressure (RFT Biomonitor; Zwönitz, Germany) and end-expiratory P<sub>CO</sub><sub>2</sub> (Heyer CO<sub>2</sub> Monitor EGM I; Bad Ems, Germany) were monitored. Arterial P<sub>2</sub>, arterial P<sub>CO</sub><sub>2</sub>, and pH were serially measured using a Compact 1 Blood Gas Analyzer (AVL Medizintechnik; Bad Homburg, Germany). Because the rats were not
paralyzed, the adequacy of the level of anesthesia was assessed by testing motor responses to tail pinch. In addition, changes of blood pressure in response to tail pinch were used to control anesthesia. Further thiopental doses (25 mg/kg ip) were applied when necessary.

A craniotomy was performed over the parietal cortex using a saline-cooled drill. A closed cranial window was implanted as previously described (9). The dura mater was removed. The craniotomy site was covered with a piece of glass cut from a coverslip. An inflow and outflow tube allowed us to superfuse the brain cortex continuously. The direct current (DC) shift was recorded using an Ag–AgCl electrode.

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Fig. 1. Experimental setup. The closed window was covered by a piece of glass cut from a coverslip. An inflow (acSFin) and outflow tube (acSFout) allowed the superfusion of the cortex with artificial cerebrospinal fluid (acSF) containing the drugs used. Cerebral blood flow (CBF) was measured by one laser-Doppler flow probe (LDF) at the window. The direct current (DC) shift was recorded using an Ag–AgCl electrode.

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Experimental Protocols

General procedures for groups 1 and 2. The drugs were first administered alone and then coapplied with increasing concentrations of ET-1 from 10 nM to 1 μM in the acSF. Equilibration time was 1 h for each application. The number of animals is given in parentheses.

ET receptor antagonists (group 1). The drugs tested were the ETa receptor antagonist BQ-123 topically applied at 500 nM (n = 7), 1 μM (n = 6), 5 μM (n = 6), and 50 μM (n = 6); the ETb receptor antagonist BQ-788 at 500 nM (n = 7), 5 μM (n = 6), and 50 μM (n = 10); BQ-123 at 500 nM together with BQ-788 at 5 μM (n = 6); and the ETa receptor agonist BQ-3020 at 1 μM (n = 6). The results of these 60 experiments were compared with 12 control experiments in which ET-1 was continuously superfused in increasing concentrations from 10 nM to 1 μM without receptor antagonist or agonist.

Phospholipase C inhibitor U-73122 and PKC inhibitor G6-6983 (group 2). To study the intracellular pathways after ET receptor activation, U-73122 in 1% DMSO was coapplied with ET-1 (10 nM–1 μM) in 10 animals and G6-6983 in 1% DMSO in another 6 animals. These results were compared with a vehicle control in which 1% DMSO was coapplied with increasing concentrations of ET-1 from 10 nM to 1 μM (n = 7).

Arachidonic acid (group 3). acSF containing arachidonic acid at a concentration of 100 μM and 1 mM was brain topically applied in six animals. The application time for each concentration was 1 h.

Chemicals and Drugs

G6-6983 was obtained from Calbiochem (Beeston/Nottingham, UK); BQ-3020 was from Alexis (Lausen, Switzerland), and all other chemicals were purchased from Sigma (Deisenhofen, Germany).

Statistical Methods

Data were analyzed by comparing relative changes of CBF and absolute changes of the DC potential. CBF changes were calculated in relation to baseline at the onset of the experiment (equal to 100%). A zero level was established at the end of the experiments after global cerebral ischemia. All data are given as means ± SD. Statistical comparisons were performed using Fisher’s exact test, ANOVA with repeated measures with Scheffé’s post hoc test or one-way ANOVA with Bonferroni’s post hoc test. A value of P < 0.05 was considered as statistically significant.

RESULTS

Systemic Variables

Systemic variables remained within physiological limits throughout the experiments. The physiological variables during the experiments are shown in Table 1.

Table 1. Physiological variables after the experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SAP, mmHg</th>
<th>Arterial Pco2, mmHg</th>
<th>Arterial Po2, mmHg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1:</td>
<td>60</td>
<td>111±16</td>
<td>36±4</td>
<td>119±12</td>
<td>7.40±0.03</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>95±8</td>
<td>33±4</td>
<td>125±12</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>Group 2:</td>
<td>23</td>
<td>106±11</td>
<td>37±4</td>
<td>119±9</td>
<td>7.41±0.03</td>
</tr>
<tr>
<td>PLC</td>
<td>6</td>
<td>99±26</td>
<td>38±2</td>
<td>116±8</td>
<td>7.38±0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n, no. of animals. SAP, systemic arterial pressure; ET, endothelin; PLC, phospholipase C.
Group 1: Receptor Profile of ET-1-Induced CSDs

ET-1 induced CSDs in a concentration-dependent manner in the absence of any ET receptor agonist or antagonist. In 2 of 12 animals, ET-1 induced CSDs at 10 nM. Ten of the animals generated CSDs at 100 nM. All animals developed CSDs when ET-1 was increased to 1 μM (shown as the control in Fig. 3, A, B, and D). CBF increased to 186 ± 65% during the first CSD. The hyperemia lasted for 86 ± 20 s. CSD was associated with a negative subarachnoid DC shift of −3.8 ± 1.8 mV lasting for 79 ± 22 s. The CSD frequency was 5 ± 3 per hour (range: 1–10). An example of original traces of the changes of CBF and DC potential during CSD in response to ET-1 is shown in Fig. 2. Similar experiments were carried out and analyzed in detail concerning CBF, intracortical and subarachnoid DC potentials, and extracellular K+ concentration ([K+]o) changes as well as propagation in a previous study (8).

To determine the ET receptor subtype involved in the generation of CSD, BQ-123, BQ-788, or BQ-3020 were brain topically coapplied with increasing concentrations of ET-1 (10 nM–1 μM). Superfusion of BQ-123 dose dependently inhibited the generation of CSDs by ET-1 (Fig. 3A). CBF increased to 159 ± 57% and DC shifted to −3.3 ± 2.8 mV during the first CSD when ET-1 and BQ-123 (500 nM) were coapplied. CSD frequency was 6 ± 4 per hour (range: 1–12). At BQ-123 (1 μM), only two of six animals generated one to five CSDs in response to ET-1. CBF increased to 173 ± 57% and DC fell to −2.9 ± 0.9 mV in response to the first CSD. Complete blockade was observed when BQ-123 was applied at a concentration of 5 μM. In addition, the ETα receptor antagonist BQ-123 dose dependently abolished the CBF decrease induced by increasing concentrations of ET-1 (ANOVA with repeated measures with the Scheffé’s post hoc test; Fig. 3A, n = 25). BQ-123, coapplied at a concentration of 50 μM, rendered ET-1 a vasodilator. This was probably related to activation of vaso-dilative ETβ receptors when the ETα receptors were blocked. Therefore, 50 μM probably represented a saturating concentration (27).

Superfusion of the ETβ receptor antagonist BQ-788 at a concentration of 500 nM and 5 μM did not inhibit the generation or changed the frequency of CSDs in response to increasing concentrations of ET-1. The hyperemia was 193 ± 67% and 216 ± 71%, respectively, during the first CSD. The negative DC shift was −2.7 ± 1.3 and −2.3 ± 1.6 mV, respectively. An inhibiting effect by BQ-788 was only achieved at a concentration of 50 μM (P < 0.01, Fisher’s exact test; Fig. 3B, n = 23). However, at this concentration, BQ-788 is no longer selective for ETβ receptors but also inhibits ETα receptors (29). The hyperemia was 204 ± 66%, and the negative DC shift was −2.5 ± 2.8 mV. The CSD frequency was 4 ± 3 per hour (range: 2–8). An interaction of BQ-788 with the ETα receptor may also explain why, at a concentration of 50 μM, BQ-788 did not augment but seemed to attenuate the ET-1-induced CBF decrease (Fig. 3B).

To study whether a combined action of ET receptors was significantly more effective than either of the ET receptors alone, BQ-123 and BQ-788 were also simultaneously applied at 500 nM and 5 μM, respectively (n = 6). This yielded a similar effect as a single application of BQ-123 at 500 nM (Fig. 3C). Therefore, a pronounced combined action of ETα and ETβ receptors was excluded.

Superfusion of the ETβ receptor agonist BQ-3020 did not induce CSDs at a concentration of 1 μM (Fig. 3D, n = 6). When BQ-3020 was coapplied with ET-1, the percentage of animals exhibiting CSDs did not change significantly (Fig. 3D, n = 6). CBF increased to 263 ± 59% during the first CSD. The associated negative DC shift was −4.5 ± 1.4 mV. The CSD frequency was 5 ± 3 per hour (range: 1–6). Interestingly, CSDs were induced by ET-1 in the presence of BQ-3020 despite increased average CBF levels compared with the controls (P < 0.05 and 0.01, respectively, two sample t-tests; Fig. 3D).

There was no statistically significant difference in frequency, hyperemic CBF level, or negative DC shift between ET-1-induced CSD in the presence or absence of BQ-123, BQ-788, and BQ-3020, respectively (one-way ANOVA with Bonferroni’s post hoc test).

Group 2: Intracellular Pathways Possibly Involved in ET-1-Induced CSDs

The signal transduction pathway initiated by activated ET receptors appears to involve stimulation of phospholipase C (PLC). PLC leads to the formation of inositol (1,4,5)-trisphosphate [Ins(1,4,5)P3] and diacylglycerol (DAG), which are the starting points for several intracellular signal cascades. One Ins(1,4,5)P3-mediated signal cascade involves an increase of the intracellular Ca2+ concentration, which in turn mediates, e.g., constriction of vascular smooth muscle (VSM) (19) or intercellular Ca2+ waves within the astroglial syncytium induced by ET-1 (63, 64). To determine the role of PLC in the process of CSD generation by ET-1, we topically coapplied the PLC inhibitor U-73122 at a concentration of 50 μM together with increasing concentrations of ET-1. U-73122 significantly reduced the percentage of animals exhibiting ET-1-induced CSDs (n = 10) compared with vehicle-treated animals (n = 7) (P < 0.01, Fisher’s exact test; Fig. 4).

DAG and intracellular calcium stimulate PKC. Therefore, we coadministered the PKC inhibitor Gö-6983 at a concentra-
ion of 10 μM together with increasing concentrations of ET-1. Gö-6983 did not affect the generation of CSDs by ET-1 (n = 6; Fig. 4).

Group 3: Does ET-1 Induce CSDs Via Release of Arachidonic Acid?

ET-1 is also known to stimulate the arachidonic acid metabolism via activation of phospholipases. For example, it has been proposed that interastroglial metabolic trafficking is inhibited via this pathway (58). Failure of energy metabolism is known to promote CSDs. Therefore, it was tested whether topical superfusion of aCSF containing arachidonic acid at a concentration of 100 μM and 1 mM induced CSD. No such effect was observed (n = 6). Arachidonic acid increased CBF to 127 ± 15% and 148 ± 30%, respectively.

DISCUSSION

General

Recently, it has been shown that the endothelial factor ET-1 is the most potent inducer of CSDs in vivo currently known (8). This finding may have particular relevance for the pathophysiological understanding of migraine, because several clinical lines of evidence suggest that, in a fraction of patients, endothelial irritation may somehow lead to migrainous aura and ET-1 may be involved in migraine (8, 60). The mechanism
by which ET-1 induces CSDs has remained enigmatic. It is not even clear which cellular target of ET-1 mediates that action. Possible cellular targets are astroglial cells, neurons, or VSM. Altered function of astrocytes could lead to CSDs, e.g., interastroglial calcium waves, altered function of neurons via a change in excitability or energy metabolism, or of VSM via a penumbra-like condition (8). That ET-1 did not induce CSD in neocortical brain slices seems to contradict the hypothesis that astrocytes or neurons are the primary targets of ET-1. That CBF only mildly decreased before ET-1-induced CSD seems to contradict VSM as the primary target (8).

**Interastroglial Communication and CSDs**

Interastroglial calcium waves have been proposed as the cellular correlate of CSDs because both phenomena can occur simultaneously and propagate at a similar rate (28, 35, 45, 46). Therefore, it is an attractive hypothesis that induction of interastroglial calcium waves by ET-1 may underlie ET-1-induced CSDs. The receptor profile of ET-1 controlling interastroglial communication is known (3): both ET\(_A\) and ET\(_B\) receptor need to be blocked to inhibit that action of ET-1. The intracellular signal transduction pathway includes PLC activation leading to formation of Ins(1,4,5)P\(_3\) production. Another product of PLC activation is DAG, DAG and intracellular calcium stimulate PKC. A role for PKC has been suggested, e.g., in ET-1-induced vascular contraction (19, 21). However, the PKC inhibitor G\(_6983\) was not able to inhibit the generation of CSD by ET-1. This finding suggests that PKC activation did not represent a critical step in the cascade leading to CSDs.

Via activation of phospholipases, ET-1 is also known to stimulate arachidonic acid metabolism. Interestingly, both arachidonic acid and ET-1 uncouple gap junctions (16, 17, 58). In vitro, uncoupling of gap junctions led to inhibition of CSD when long-chain alkyl alcohols were applied (35, 46), whereas another potent uncoupling agent, carbeneoxolone, had no effect on CSDs (65). The discovery that a potent gap junction blocker such as ET-1 is a highly potent inductor of CSDs is indeed surprising. A possible explanation could be related to a disturbance of interastroglial metabolic trafficking via gap junction blockade. Thus, with the use of a scraped monolayer of cultured astrocytes, it was shown that arachidonic acid at 50 \(\mu\)M blocked the diffusion of radioactively labeled glucose, glucose-6-phosphate, and lactate through gap junctions similarly to ET-1 at 100 nM, octanol at 600 \(\mu\)M, or \(\alpha\)-glycyrrhetinic acid at 200 \(\mu\)M (17, 58). In vivo, but not in brain slices, inhibited metabolic trafficking could interfere with neuronal nutrition because astrocytes may constitute a physical barrier in the supply of metabolic substrates from blood to neurons (49). A decreased supply of energy substrates is known to promote CSDs. In addition, CSD-like depolarizations under the condi-

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**Fig. 4.** The signal transduction pathway initiated by ET receptors has been shown to involve phospholipase C (PLC) in both vascular smooth muscle and astrocytes. It is shown that the PLC inhibitor U-73122 significantly inhibited CSD generation by ET-1 compared with the vehicle control \((p < 0.05)\). In contrast, the PKC inhibitor G\(_6983\) had no significant effect.
tion of energy compromise are less sensitive to gap junction blockade with long-chain alkyl alcohols (1). Earlier, it was already suggested that arachidonic acid could be involved in the mechanism underlying CSDs (39). However, we found that arachidonic acid, even at high concentrations, did not induce CSDs in vivo, in contrast to ET-1. This finding did not support the hypothesis that inhibited metabolic trafficking was critically involved in ET-1-induced CSDs.

In addition to these findings specifically related to ET-1-induced CSD, there are several more general arguments against interastroglial communication being crucial for the generation or propagation of CSDs (for a review, see Ref. 56). Thus Basarkey et al. (2) showed that CSDs in calcium-free medium also occurred in the absence of intercellular calcium waves. In addition, metabolic poisons for astroglial cells, fluorocitrate and fluorocitrate, did not prevent CSDs but rather facilitated their onset (37).

The increase of CBF by arachidonic acid may be related to the formation of epoxyeicosatrienoic acids by astrocytes (23) or direct activation of tandem pore domain potassium channels in VSM (6).

CSD-Like Depolarizations and a Penumbra-Like Condition

Because ET-1 is a potent vasoconstrictor, it appears possible that CSD-like depolarizations are triggered through an ischemic penumbra-like condition. This hypothesis has been extensively discussed in an earlier study (8). In brief, arguments in support were 1) that ET-1 did not induce CSD in brain slices; 2) that small increases in \( K^+ \) preceded ET-1-induced CSDs similar to those observed before CSD-like depolarizations in an ischemic penumbra (43, 47); and 3) that a nitric oxide (NO) donor shifted the threshold concentration of ET-1 initiating CSDs to a higher level, whereas neither initiation nor propagation of CSDs triggered by needle stab was affected by a NO donor (32). The main argument against the vascular theory was that ET-1 decreased CBF only slightly before it induced CSDs (8). In the present study, we showed that ET-1 even induced CSDs even though CBF was above baseline when we coapplied the ET \(_A\) receptor agonist BQ-3020. An earlier report, using topical application of ET-1 to the brain surface, consistently showed that the threshold concentration of ET-1 producing ischemia in a large tissue volume is above the range that, in our model, led to CSDs (15). However, the laser-Doppler flow probe measures CBF in a rather large volume of \( \sim 1 \text{ mm}^3 \). It is possible that the flow distribution in the tissue is heterogeneous and a very small ischemic region, e.g., at the cortical surface, remains undetected. In our study, the receptor profile of ET-1-induced CSDs is consistent with that of the vasoconstrictor action of ET-1. Therefore, in contrast to the astroglial theory, it remains a possibility that the vascular action of ET-1 is involved in the generation of CSDs. This issue has to be addressed in a future study. Another vascular hypothesis to investigate is related to the question of whether disruption of the blood-brain barrier (BBB) by ET-1 may promote the induction of CSD. Consistently, similar to vasconstrictor, BBB opening by ET-1 is mediated via ET \(_A\) receptors (44, 57). Furthermore, it has recently been shown that BBB disruption causes changes in neuronal excitability (53). It deserves further study as to whether these changes are relevant for the initiation of CSDs by ET-1. Alternatively to the vascular hypothesis, the receptor profile of ET-1-induced CSDs leaves the possibility that ET-1 triggers CSDs via a direct action on neurons, e.g., related to augmentation of glutamergic neurotransmission (54).

In general, in this discussion, when comparing the CSD threshold in response to ET-1 with other ET-1-related effects, it is necessary to consider the influence of different anesthetics in vivo. For example, thiopental is known to reduce both neuronal metabolism and baseline CBF (11). These actions possibly lower the CSD threshold. In contrast, other anesthetics may increase the CSD threshold either by inhibiting CSDs directly or by antagonizing ET-1. Halothane is such a candidate. It is known to inhibit ET-1 effects directly such as vasoconstriction in rat aortic rings or astroglial Ca\(^{2+}\) increases in cell culture (4, 63). In addition, it seems to directly interfere with CSD initiation (51). These issues have to be addressed in a future study.

Relation Between ET-1- and K\(^+\)-Induced CSDs

Leão (40) described CSDs in 1944 as a wave of depression of "spontaneous" electrocorticographic activity that propagated in the rabbit cortex. Several theories on the mechanism of CSD initiation and propagation later evolved [reviewed by Somjen (56)]. Grafstein (20) and van Harreveld and Fifkova (62) coined the two most influential theories: the "potassium" and "glutamate" theories. Grafstein proposed that K\(^+\) released from neurons during intensive firing accumulates in the extracellular space. This increase in interstitial K\(^+\) would further depolarize the firing neurons leading to a vicious circle resulting in the depression of neuronal excitability. Simultaneously, the released K\(^+\) would diffuse to neighboring neurons, there giving rise to the cycle of depolarization followed by neuronal inactivation. Although some aspects of this theory such as a critical role for action potential firing were not confirmed in later studies, the core of the theory has survived. Particular evidence exists that an increase in interstitial K\(^+\) is important in the ignition and evolution of CSD rather than its propagation (56). The van Harreveld glutamate theory was based on the findings that this amino acid can induce CSDs, it is released during CSDs and the Na\(^+\) channel blocker tetrodotoxin suppresses action potential firing but neither inhibits CSD nor glutamate release during CSDs nor glutamate's excitatory action. The glutamate theory was later supported by the observation that \( \tilde{N} \)-methyl-D-aspartate (NMDA) receptor antagonists inhibit CSD propagation in normal tissue (56).

The very initial cascade of ET-1- and K\(^+\)-induced CSDs must be different because ET-1 does not induce CSDs in brain slices, in contrast to K\(^+\). However, there are also similarities between the two inductors of CSDs. Thus the repolarization of K\(^+\)-induced CSDs is prolonged at the initiation site both in vivo and in vitro similar to the repolarization of ET-1-induced CSDs in vivo (8). Second, K\(^+\)-induced CSD is preceded by an artificial increase in baseline [K\(^+\)], at the initiation site; ET-1-induced CSD is also preceded by a gradual rise in baseline [K\(^+\)]. Although this occurs spontaneously, similarly as in hypoxic CSD in brain slices (43) or periinfarct depolarizations in vivo (47). Hypothetically, the combination of a rise in baseline [K\(^+\)] preceding CSD and a prolonged repolarization phase represent two key features characterizing the initiation site of CSD in general, independent of the CSD-inducing
agent. Consistent with a distinct pathogenetic situation between initiation and propagation sites, NMDA receptor agonists less potently inhibit initiation of CSD than its propagation in surrounding normal tissue (56). The gradual rise in baseline \([K^+]_o\) at the initiation site may be related to opening of \(K^+\) channels (43) and downregulation of the \(Na^+\)-\(K^+\)-ATPase activity (10), whereas the latter may also be responsible for the delay in repolarization.

ET-1 causes a small rise in baseline \([K^+]_o\) in vivo but not in brain slices before CSD. This is again consistent with a link between the gradual rise in baseline \([K^+]_o\) and the initiation of CSD as discussed above. On the basis of this notion, we speculate that the key for understanding the mechanism underlying ET-1-induced CSD is hidden in the process by which ET-1 causes the gradual rise in baseline \([K^+]_o\). Consistent with the above hypothesis regarding \(Na^+\)-\(K^+\)-ATPase, we have recently shown that ET-1 downregulates \(Na^+\)-\(K^+\)-ATPase activity in vivo even before CSD starts (50). This mainly occurs on account of the \(\alpha/\beta\) activity. The reasons are unknown for the decrease in \(Na^+\)-\(K^+\)-ATPase activity. Possibly, perturbations in membrane integrity and/or association of the \(\alpha\)-isoforms of this enzyme are responsible (30).

In conclusion, based on the identified ET receptor profile in this study, we conclude that either VSM cells or neurons, but not astroglial cells, constitute the likely primary targets of ET-1 to induce CSD. Following this conclusion, ET-1 would depolarize neurons either via direct action or as a consequence of decreased energy supply due to contracted VSM. The special features of ET-1 may be particularly interesting with respect to clinical conditions that often present with both migraine aura and stroke, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (7) or hereditary infantile hemiparesis, retinal arteriolar tortuosity, and leukoencephalopathy (61).

**REFERENCES**

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**REFERENCES**


