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(S)-Emopamil Attenuates Acute Reduction in Regional Cerebral Blood Flow Following Experimental Brain Injury

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ABSTRACT

We examined the effects of (S)-emopamil, a phenylalkylamine calcium channel blocker with serotonin receptor antagonist properties, on regional cerebral blood flow (rCBF) following experimental brain injury in the rat. Animals were subjected to fluid percussion brain injury of moderate severity (2.1 atm), and received (S)-emopamil (20 mg/kg, i.p., $n = 10$) or saline ($n = 10$) at 20 minutes postinjury and 2.5 hours after the first injection of the drug. Consecutive rCBF measurements were performed: (1) prior to injury, (2) 15 minutes, (3) 90 minutes, and (4) 4 hours postinjury, using the radiolabeled microsphere technique. Brain injury produced an acute and significant reduction of rCBF at 15 minutes postinjury in all the regions examined ($p < 0.05$). At 90 minutes postinjury, rCBF remained significantly depressed in the forebrain regions. All brain regions showed a recovery of rCBF to normal by 4 hours following injury in saline-treated animals, with the exception of injured left parietal cortex and bilateral hippocampi, where rCBF remained significantly depressed. A significant attenuation of the trauma-induced reduction in rCBF was observed at 70 minutes after the first administration of (S)-emopamil in the forebrain regions and cerebellum ($p < 0.05$). Following the second (S)-emopamil injection, the significant improvement in rCBF observed in left injured cortex was maintained. These results suggest that (S)-emopamil may be efficacious in reversing post-traumatic alterations in rCBF, which may contribute to the post-traumatic pathophysiologic sequelae.

INTRODUCTION

It has been suggested that cerebral ischemia may be one of the most important mechanisms in the production of secondary or delayed brain damage following traumatic brain injury (Bouma et al., 1991; Miller, 1985). Postmortem studies have indicated that a high incidence of histopathologic ischemic brain damage occurs after severe head injury (Graham et al., 1978, 1989). Experimental studies have also postulated that the decrease in regional cerebral blood flow (rCBF) following brain injury might contribute to the development of

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secondary brain injury (Yamakami and McIntosh, 1989, 1991; Yuan et al., 1988). In recent studies of brain ischemia, calcium channel blockers have been shown to increase rCBF and preserve brain metabolism after cerebral ischemia in rats (Bielenberg et al., 1987; Jacewicz et al., 1990; Szabo and Hofmann, 1989). It is conceivable then that secondary injury following brain trauma might, in part, be attenuated by calcium channel blockers through preservation of CBF and restoration of brain metabolic function.

(S)-emopamil is a novel phenylalkylamine calcium channel blocker developed especially for central nervous system (CNS) penetration. This compound has a high blood-brain barrier permeability and exhibits high affinity for L-type calcium channels (Szabo, 1989). It has been previously reported that (S)-emopamil increases rCBF and accelerates the restoration of high energy phosphates after cerebral ischemia in rats (Bielenberg et al., 1987; Szabo and Hofmann, 1989). (S)-emopamil has also been shown to reduce infarct size following middle cerebral artery occlusion in rats (Nakayama et al., 1988) and to protect hippocampal neurons from ischemic injury after global brain ischemia in rats (Lin et al., 1990). In addition to its calcium channel antagonist properties, (S)-emopamil is a potent antagonist of serotonin-2 receptors (Szabo, 1989). Since serotonin has been suggested to play a role in ischemic CNS injury by constricting blood vessels and increasing vascular permeability after ischemia (Defeudis, 1989), spinal trauma (Liu et al., 1990), and brain injury (Pappius, 1991), (S)-emopamil may also have a protective effect on cerebral circulation after brain trauma. To this end, we have recently demonstrated that (S)-emopamil reduces regional cerebral edema, attenuates post-traumatic cognitive dysfunction, and improves functional motor recovery following fluid percussion brain injury in the rat (Okiyama et al., 1992). The present study was undertaken to explore the potential mechanism by which (S)-emopamil may exert its neuroprotective effects and clarify the effect of (S)-emopamil on regional CBF following experimental brain injury in the rat, utilizing the radiolabeled microsphere technique.

MATERIALS AND METHODS

Surgical Preparation

Male Sprague-Dawley rats (350–400 g, $n = 23$) were anesthetized with isoflurane and artificially ventilated with a mixture of isoflurane (1.5–2%), oxygen, and air, using a Harvard rodent ventilator (Harvard Inc., South Natick, MA) and a veterinary anesthesia machine with a Forane vaporizer (Ohio Medical Products, Madison, WI). Core body temperature was maintained at 36.5–37.5°C using a heating pad. We did not monitor brain temperature since, in our previous study, no significant changes in temporalis muscle temperature were observed between control and (S)-emopamil treated groups (Okiyama et al., 1992). A catheter (PE-50) was placed in the right femoral artery for blood pressure recording, blood sampling (for $PaCO_2$, PaO_2, pH, and hematocrit determinations), and collection of the arterial reference sample. The left ventricle of the heart was cannulated via the right subclavian artery; a cannula (Bolab Vinyl Tubing, size v/1, [inner diameter 0.28 mM], connected to v/3 [inner diameter 0.58 mM], Bolab Products, Lake Havasu City, AZ) was inserted into the subclavian artery at 1–2 mM proximal to the trifurcation of the vessel at the level of the brachial plexus and advanced for 30–33 mm. During the ventricular cannulation, pressure pulses derived from the catheter were continuously monitored to insure the proper placement of the ventricular catheter.

Experimental Brain Injury

Experimental brain injury was induced using the lateral (parasagittal) fluid percussion model (McIntosh et al., 1989). The injury device was connected to the animal via a Luer-Lock fitting, which was rigidly fixed with dental cement to the animal's skull through a craniotomy centered over the left parietal cortex. The device produces a pulse of increased intracranial pressure (ICP) of 21–23 msec duration through the rapid injection of saline into the closed cranial cavity, resulting in brief displacement and deformation of neural tissue. This pressure pulse (atmosphere) is measured extracranially by a transducer (Gould, Inc.) housed in the injury device.

Experimental Protocol

After surgical preparation isoflurane concentration was adjusted to 1%, and at least 60 minutes were allowed for stabilization of the alveolar gas concentration before the first (preinjury) microsphere injection. PaO_2 and $PaCO_2$ were maintained in the range of 70–110 mMHg and 35–45 mMHg, respectively (see Results section). All animals were subjected to fluid percussion brain injury (over the left parietal cortex) of moderate severity (2.1 atm). Twenty minutes postinjury, animals randomly received 1 ml of either (S)-emopamil (20 mg/kg i.p., $n = 10$), or saline (equal volume i.p., $n = 10$). In each of these groups, a second dose identical to the first was administered 2.5 hours following the first dose of (S)-emopamil or saline. Doses and schedule of drug administration were based on consultation with Knoll AG Pharmaceutical scientists and previous studies with the compound (Nakayama et al., 1988; Okiyama et al., 1992). Sham-operated uninjured animals received the same preparation and surgery and were treated with (S)-emopamil (20 mg/kg i.p., $n = 3$) according to the same treatment paradigm described above.

Regional CBF Measurement

In the present experiment, we used microspheres (15 μM), labeled with either ^{113}Sn, ^{95}Nb, ^{46}Sc, or ^{57}Co (NEN Research Products, E.I. duPont de Nemours & Co., Inc.). Four consecutive injections of microspheres labeled with each radionuclide were performed in a random fashion: (1) prior to injury, (2) 15 minutes, (3) 90 minutes (70 minutes after the first treatment), and (4) 4 hours (70 minutes after the second treatment) following brain injury. Microspheres, suspended in isotonic saline with 0.01% Tween-80 and vortexed for 1 minute, were injected into the left ventricle via the ventricular catheter (1 ml, 100,000 microspheres per injection) over 45 seconds. Beginning 15 seconds prior to each microsphere injection, a reference blood sample was withdrawn continuously at a rate of 0.4 ml/min (total withdrawal time = 2.5 minutes), using a Harvard infusion-withdrawal pump (Harvard Apparatus Co., Milton, MA). The blood loss from each reference blood sampling was replaced immediately with 1 ml of venous blood taken from a donor rat, anesthetized with pentobarbital (60 mg/kg, i.p.). After the fourth microsphere injection, animals were sacrificed by decapitation. The brains were rapidly removed and dissected into: injured left parietal cortex, contralateral right parietal cortex, bilateral hippocampi, thalamus, brainstem, and cerebellum. The radioactivity of each tissue sample and each reference blood sample was counted on an AUTO-GAMMA RIA Systems Model 500 (Packard Instrument Co., Inc., Downers Grove, IL), and the radioactivity for each nuclide was determined by means of a least-squares radionuclide separation technique (Baer et al., 1984). CBF was calculated from the equation

$$\text{CBF (ml/min/100 g)} = \{[\text{counts in brain sample}] \times [\text{withdrawal rate of reference blood (ml/min)}] / [\text{counts in reference blood sample}] / [\text{weight of brain sample (g)}]\} \times 100$$

Data Analysis

All data are expressed as means ± standard deviation (S.D.). Cardiovascular variables, blood flow data across groups were examined using analysis of variance (ANOVA) followed by Student-Newman-Keuls test, and continuous variables subjected to repeated measurements in the same group were assessed by repeated-measures ANOVA followed by Bonferroni t test. A p value of less than 0.05 was considered statistically significant.

RESULTS

Physiological Variables

No significant hemodynamic changes were observed following four repeated injections of microspheres, in mean arterial blood pressure (MABP), heart rate, hematocrit, and arterial blood gases in saline treated traumatized animals (Fig. 1, Table 1). Animals treated with (S)-emopamil showed an immediate fall in

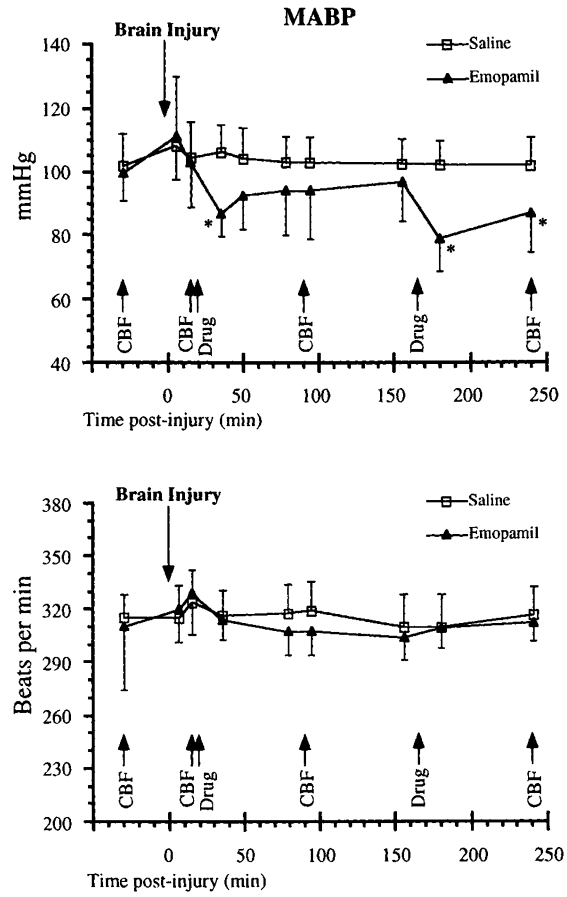

FIG. 1. Changes in **(A)** mean arterial blood pressure (MABP) and **(B)** heart rate following fluid-percussion brain injury and treatment with (S)-emopamil or saline. Data are expressed as mean value. Error bars represent standard deviations. CBF = rCBF measurement; Drug = treatment with (S)-emopamil or saline; Saline = saline-treated animals (n = 10); Emopamil = (S)-emopamil–treated animals (n = 10). * = $p < 0.05$, compared to saline-treated animals.

TABLE 1. PHYSIOLOGIC VARIABLES

Time postinjury	*Preinjury*	*15 Minutes*	*90 Minutes*	*4 Hours*
PaO_2 (mMHg)				
Saline	102.5 ± 23.3	118.6 ± 23.6	106.6 ± 29.8	109.3 ± 19.2
(S)-emopamil	106.0 ± 26.9	100.9 ± 26.3	91.6 ± 13.4	92.3 ± 14.2
$PaCO_2$ (mMHg)				
Saline	38.9 ± 2.1	40.8 ± 2.1	40.2 ± 3.5	40.8 ± 2.1
(S)-emopamil	36.9 ± 3.5	39.8 ± 3.2	40.5 ± 2.5	39.8 ± 3.3
pH				
Saline	7.36 ± 0.05	7.35 ± 0.04	7.37 ± 0.06	7.34 ± 0.04
(S)-emopamil	7.39 ± 0.03	7.37 ± 0.04	7.34 ± 0.03	7.36 ± 0.03
Hematocrit (%)				
Saline	44.6 ± 1.1	45.2 ± 1.9	43.8 ± 2.4	43.0 ± 2.4
(S)-emopamil	43.0 ± 4.2	43.1 ± 4.2	42.9 ± 3.8	43.0 ± 2.9

Data are expressed as mean ± S.D. Saline = saline-treated animals (n = 10); (S)-emopamil = (S) emopamil–treated animals (n = 10).

TABLE 2. rCBF IN SHAM-OPERATED UNINJURED ANIMALS FOLLOWING TREATMENT WITH (S)-EMOPAMIL

rCBF Measurement	*First (Control)*	*Second (Control)*	*Third*[a]	*Fourth*[b]
Cerebral cortex[c]	132 ± 17	127 ± 17	174 ± 37	124 ± 23
Hippocampus[c]	145 ± 34	147 ± 24	172 ± 18	122 ± 21
Thalamus	108 ± 23	98 ± 17	131 ± 49	101 ± 22
Brain stem	105 ± 23	108 ± 11	130 ± 31	116 ± 10
Cerebellum	102 ± 15	111 ± 40	140 ± 15	108 ± 16
Total brain	126 ± 25	117 ± 16	152 ± 31	105 ± 24

Data are expressed as mean value (ml/100g/min) ± S.D. ($n = 3$).

[a,b] rCBF measured at 70 min after the first and second (S)-emopamil treatments, respectively.

[c] Bilateral cortices or hippocampi.

MABP following the first drug administration ($p < 0.05$), which quickly returned to normal and remained slightly below control values by the time of third CBF measurement ($p > 0.05$). Following the second (S)-emopamil injection, however, a significant decrease in MABP was observed, which remained significantly suppressed at the time of fourth CBF measurement ($p < 0.05$, Fig. 1). No significant differences were observed in heart rate, PaO_2, $PaCO_2$, pH, and hematocrit in treated versus control animals (Table 1).

Regional Cerebral Blood Flow

After the first administration of (S)-emopamil in sham operated uninjured animals ($n = 3$), a trend toward an increase in rCBF was observed in all regions (Table 2). Regional CBF returned to normal after the second bolus of (S)-emopamil. Table 3 presents both absolute and percent change in rCBF postinjury, while relative rCBF (expressed as percent change from control rCBF values) is presented for the evaluation of the time course of rCBF changes and pharmacological effects on CBF after brain injury (Figs. 2 and 3). Saline-treated brain-injured animals showed a marked reduction of rCBF at 15 minutes postinjury in all the regions examined ($p < 0.05$). At 90 minutes postinjury, rCBF remained significantly depressed in all brain regions ($p < 0.05$) except hindbrain (brainstem and cerebellum). By 4 hours following injury, rCBF had recovered to normal levels in most regions, except the injured left parietal cortex and bilateral hippocampi, where rCBF remained

TABLE 3. ABSOLUTE AND PERCENT OF CONTROL CHANGES IN rCBF AT 15 MIN FOLLOWING FLUID-PERCUSSION BRAIN INJURY

	Saline			*(S)-emopamil*		
	Preinjury	*15 Min*	*% of Control*	*Preinjury*	*15 Min*	*% of Control*
LC	156 ± 10	85 ± 13[a]	54	153 ± 20	83 ± 7[a]	54
RC	171 ± 18	86 ± 12[a]	50	173 ± 20	82 ± 7[a]	47
LH	168 ± 20	85 ± 11[a]	51	167 ± 15	83 ± 13[a]	49
RH	173 ± 26	78 ± 15[a]	45	175 ± 22	81 ± 10[a]	46
TH	143 ± 19	81 ± 7[a]	57	151 ± 13	84 ± 11[a]	56
BS	190 ± 11	103 ± 5[a]	54	185 ± 16	98 ± 12[a]	53
CB	147 ± 13	98 ± 5[a]	67	137 ± 7	89 ± 8	65
Total brain	164 ± 41	84 ± 20†	54	162 ± 36	87 ± 30†	53

Data are expressed as mean ± standard error of the mean. Absolute data are indicated as: ml/100g/min. Saline = saline-treated animals ($n = 10$); (S)-emopamil = (S)-emopamil–treated animals ($n = 10$); LC = left injured parietal cortex; RL = right injured parietal cortex; LH = left hippocampus; RH = right hippocampus; TH = thalamus, BS = brain stem; CB = cerebellum.

[a] $p < 0.05$, compared to preinjury control value.

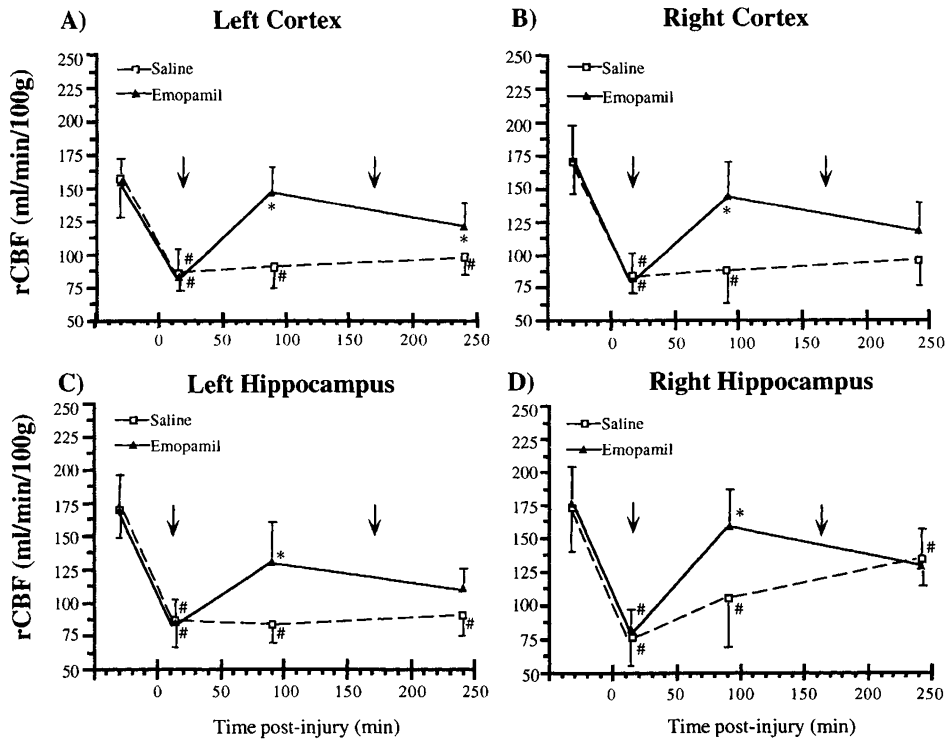

FIG. 2. A–D. Changes in rCBF following fluid-percussion brain injury and treatment with (S)-emopamil or saline. Error bars represent standard error of the mean. Arrows represent administration of (S)-emopamil or saline. Saline = saline treated animals (n = 10); Emopamil = (S)-emopamil–treated animals (n = 10). # = $p < 0.05$, compared to pre-injury baseline value. * = $p < 0.05$, compared to saline-treated animals.

significantly depressed ($p < 0.05$). Of interest, the most profound and persistent focal reduction in rCBF occurred in the left hippocampus (ipsilateral to the site of maximal cortical injury).

Following administration of (S)-emopamil at 20 minutes postinjury, a marked improvement of rCBF value was observed 90 minutes postinjury in all the forebrain regions (bilateral cortices, hippocampi, and thalamus) and cerebellum ($p < 0.05$) (Figs. 2 and 3). Among these regions, injured left cortex showed the greatest increase in rCBF (71.3% increase), although the degree of increase was not significantly greater than that in the other regions (Fig. 2B). In the brainstem, no significant difference was observed in rCBF between saline and (S)-emopamil treated groups (Fig. 3B). Following the second drug administration, a significant improvement of rCBF (59% increase; $p < 0.05$) was maintained in left injured cortex. However, no significant differences between saline and drug-treated groups were observed in the other brain regions examined. There was a trend toward decrease in rCBF in all the regions examined after the second (S)-emopamil treatment, when compared to the rCBF values obtained during the third measurement (after the first (S)-emopamil treatment), though this change in rCBF was not statistically significant.

DISCUSSION

The present study demonstrates that fluid percussion brain injury in rats produced an immediate, widespread, and marked reduction of rCBF. This reduction was significantly reversed following treatment with (S)-emopamil. The observed post-traumatic changes in rCBF are identical to those previously reported in this model (Yamakami and McIntosh, 1989, 1991).

In rats, three repeated administrations of 100,000 microspheres per injection have been reported to cause no significant change in blood pressure, cardiac output, or tissue blood flow (Hoffmann et al., 1981; Yamakami and McIntosh, 1989). Stanek et al. (1983) have also shown that no hemodynamic changes result until

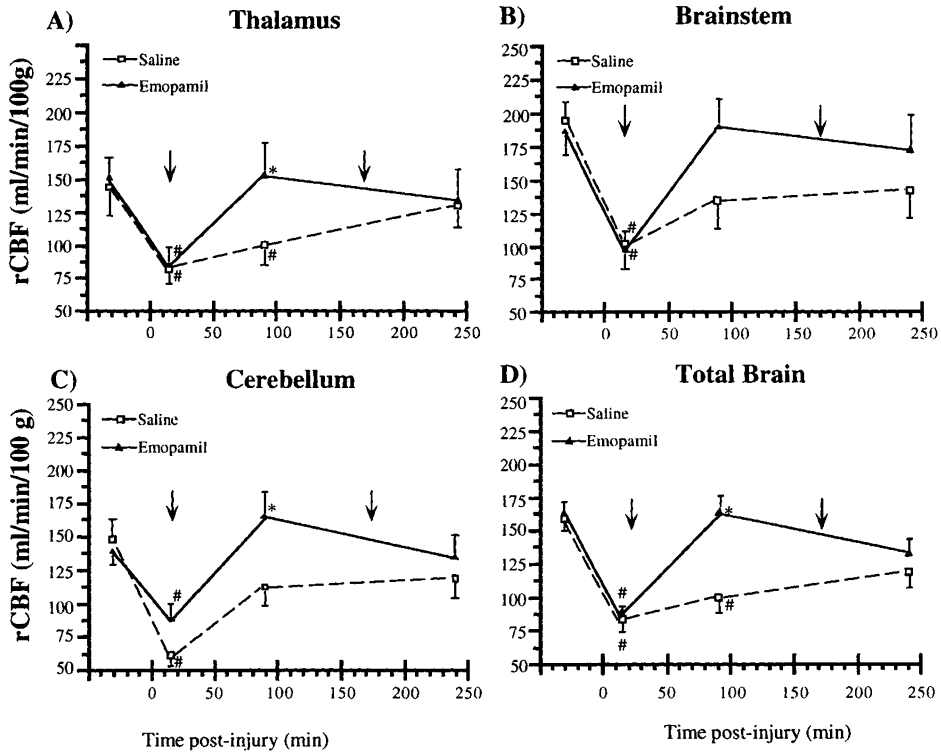

FIG. 3. (A–D). Changes in rCBF following fluid-percussion brain injury and treatment with (S)-emopamil or saline. Error bars represent standard error of the mean. Arrows represent administration of (S)-emopamil or saline. Saline = saline-treated animals (n = 10); Emopamil = (S)-emopamil–treated animals (n = 10). # = $p < 0.05$, compared to preinjury baseline value. * = $p < 0.05$, compared to saline-treated animals.

1,440,000 microspheres have accumulated in the rat. In the present study we used four consecutive injections of 100,000 microspheres per injection (total 400,000), with the blood loss being replaced by blood transfusion from donor rats. As a consequence, no significant hemodynamic changes were observed in the saline treated injured rats (Table 1). In our study the smallest tissue samples were the hippocampi, (weighing 55–65 mg each), which contained the minimum number of microspheres, conservatively estimated at 300–500, while larger samples from other regions all contained greater than 500 microspheres (results not shown). Although variability was observed in all regional rCBF determinations, tissue samples containing greater than 400 microspheres have been shown to be sufficient for accurate measurements (Buckberg et al., 1972). In our preliminary study the rCBF values in each animal were consistent over the four microsphere injections (data not shown). In the uninjured drug-treated group, each animal also showed consistent flow data over the first two flow measurements. While the absolute control (preinjury) rCBF values were variable, percent change from control value in each time point was more consistent among animals. One of the reasons for the variability between animals may be the relatively wide range of $PaCO_2$ (35–45 mMHg) between experimental animals in this study. It must be noted that one limitation of the radiolabeled microsphere technique lies in the fact that the relationship between variability and microsphere number is logarithmic, with accuracy of measurement decreasing rapidly as tissue sample microsphere concentration falls. Given the small size of the rat hippocampus, our values of cerebral blood flow in this region may actually approach the lower level of detectability for this technique. They also most likely reflect flows that are accurate and valid. We are confident moreover, that the changes (relative to baseline) observed in hippocampal blood flow following brain injury and drug treatment are accurate using the microsphere technique.

Since isoflurane has minimal effects on cerebral circulation under 1.0 minimum alveolar concentration

level (i.e., 1.45% in inspired gas concentration) (Eger, 1980; Todd and Drummond, 1984; Maekawa et al., 1986), we used 1% isoflurane (inspired gas concentration) for anesthesia during CBF measurements. Accordingly, the rCBF data observed in this study are unlikely to have been disturbed by the effect of anesthesia.

Similar to the present study, several clinical studies have recently reported a significant reduction in regional or global CBF within the first few hours after severe brain injury, and have suggested that this decreased perfusion might have important effects on neurologic status and outcome (Bouma and Muizelaar, 1992; Marion et al., 1991). The absolute post-traumatic rCBF values in the present study were higher than the established ischemic threshold for infarction, since the CBF threshold for irreversible histologic damage (i.e., infarction) in primates and cats has been reported to lie between 10 and 18 ml/100 g/min (Astrup, 1982; Heiss, et al., 1976; Jones et al., 1981; Siesjo, 1992; Symon, (1985). However, in recent studies of rCBF in the rat, the basal resting CBF has been reported to be 100–180 ml/100 g/min (Goldman et al., 1991; Jacewicz, et al., 1992; Maekawa et al., 1986; Memezawa et al., 1992; Mies et al., 1991; Yamakami et al., 1989, 1991; Yuan et al., 1988), which is approximately threefold higher compared to the primate (33–55 ml/100 g/min) (Astrup et al., Jones et al., 1981; Symon et al., 1974). In addition, CBF of 40–60 ml/100 g/min in the rat has been reported to be associated with several pathologic changes, including inhibition of protein synthesis (Mies et al., 1991), the expression of heat shock protein (Jacewicz et al., 1986), and an infarction in spontaneously hypertensive rats (Jacewicz et al., 1992). Therefore, Jacewicz et al. (1992) have suggested that the relative threshold (percent change from normal rCBF values) may be more uniform across the mammalian species, and have proposed the CBF threshold for infarction as 30–40% of basal CBF. It has also been demonstrated that morphologic changes after ischemia occur in areas where the reduction in CBF is approximately 50% of the control value (Mendelow et al., 1984). Although the absolute rCBF values were high, the present study demonstrated an acute post-traumatic rCBF reduction up to 43% of basal rCBF, which might, in part, contribute to the development of previously characterized selective hippocampal neuronal cell loss (Cortez et al., 1989), electrophysiologic changes (Lowenstein et al., 1992), cognitive dysfunction (Smith et al., 1991), and neurologic motor dysfunction (McIntosh et al., 1989). The mechanism by which (S)-emopamil attenuated both memory and motor dysfunction following FP brain injury (Okiyama et al., 1992) may be related to its ability to reverse this post-traumatic reduction in rCBF.

A number of studies suggest that traumatic and ischemic brain injury share common pathological sequelae that contribute to delayed neuronal damage, including an activation of excitatory amino acid neurotransmitters (Faden et al., 1989; Jenkins et al., 1989; McIntosh et al., 1992). It has also been shown that experimental brain injury increases the vulnerability of traumatized brain to secondary ischemia, which may be mediated through synaptic modifications induced by excitatory neurotransmitters (Jenkins et al., 1989). Consequently, alterations in CBF, even those above the ischemic flow threshold, could provoke infarction if the insult is associated with brain trauma.

Several recent studies have suggested that post-traumatic hypoperfusion might be a secondary result of primarily impaired metabolism via normal flow-metabolism coupling, and reduced blood flow may not necessarily indicate ischemia (Bouma and Muizelaar, 1992; Obrist et al., 1984). However, although several investigators have suggested that brain trauma might produce disturbances of cerebral oxidative metabolism (Duckrow et al., 1981; Hovda et al., 1991; Unterberg et al., 1988; Vink et al., 1987), mitochondrial energy production has been reported to be quite resistant to fluid-percussion brain injury (Duckrow et al., 1981; Vink et al., 1990). Conversely, using [^{14}C]2-deoxyglucose autoradiography, Yoshino et al. (1991) have reported that following experimental fluid percussion brain injury in the rat, a hypermetabolic response occurs as early as 30 minutes postinjury, which is resolved by 6 hours. It has also been demonstrated that a remarkable increase in the local glucose utilization occurs immediately following experimental subdural hematoma (Kuroda et al., 1992). Both studies have suggested that excitatory neurotransmission may play a major role in the development of post-traumatic hypermetabolism. After brain injury, intra- and extracellular ion balance are disrupted by ionic shifts through transmitter-gated ion channels. The post-traumatic increases in energy demand may reflect the efforts of cells to restore normal ionic homeostasis (Katayama et al., 1990). This acute increase in glucose utilization and energy demand, coupled with a global hypoperfusion may result in a relative metabolism perfusion mismatch, which might have some effects on the development of post-traumatic secondary brain injury, either by inducing substrate depletion, disturbances in potassium clearance from brain tissue, or disturbances in clearance of metabolites such as lactate.

Although a tight coupling of normal neuronal activity and blood flow has been demonstrated (Kuschinsky and Wahl, 1978; Sokoloff, 1981), a coupling of regional CBF and oxygen metabolism is not necessarily observed during phasic neural activity, including physiologic neural stimulation (Fox et al., 1988), rapid-eye-movement (REM) sleep (Lydic et al., 1991; Madsen et al., 1991), and epileptic seizure (Ackerman and Lear, 1989; Penfield, 1937; Plum and Posner, 1968). These studies suggest that neural stimulation preferentially activates glycolytic glucose metabolism rather than oxidative glucose metabolism, associated with concomitant increase in rCBF. The disproportionate changes in CBF compared to oxygen metabolism might also take place in the pathologic state such as traumatic brain injury, and CBF may not necessarily change in relation to the change in oxygen metabolism.

Several factors have been postulated to contribute to the post-traumatic acute reduction of rCBF: Cerebral vasospasm has been clinically demonstrated after head injury, using cerebral angiography (Suwanwela and Suwanwela, 1972) and transcranial doppler ultrasonography (Weber et al., 1990), and is probably one of the possible explanations of post-traumatic hypoperfusion. Pathologic changes in cerebral microvasculature has also been implicated in secondary ischemia after brain trauma. It has been shown that perivascular podocytic process swelling in astrocytes following severe head trauma may cause the compression of the microcirculation, resulting in a reduction in local tissue perfusion (Maxwell et al., 1988; Bullock et al., 1991). Abnormalities in the pial vessels, including endothelial damage, have been found after experimental brain injury, suggesting that the sudden dilation of the cerebral vessels, produced by a sympathetic-induced rise in blood pressure, causes multiple endothelial lesions (Wei et al., 1980). The endothelial injury and dysfunction may lead to impaired endothelium-dependent vasodilation, resulting in hyperactivity of underlying vascular smooth muscle and pathophysiologically elevated vascular tone (i.e., vasospasm) (Rubanyi, 1991).

Cerebrovascular circulation is normally regulated tightly to maintain constant perfusion of the brain (Paulson et al., 1990). However, calcium channel antagonists have been shown to dilate the cerebral resistance vessels and increase CBF (Mohamed et al., 1984; Vorstrup et al., 1986). In addition, the calcium antagonist nimodipine has been reported to impair autoregulation via a reduction in blood pressure (Harris et al., 1982). The first administration of (S)-emopamil in sham-operated animals produced a trend toward increased rCBF in all regions, while rCBF returned to normal after the second bolus of (S)-emopamil (Table 2, not significant). This trend toward decreased rCBF following the second bolus of this compound was also observed in the traumatized animals, though this change in rCBF was not statistically significant. These results suggest that (S)-emopamil may cause cerebral vasodilation, with subsequent impairment of cerebrovascular autoregulation to decreased arterial pressure. Consequently, (S)-emopamil-induced hypotension observed during the last rCBF measurement (Fig. 1) might have offset the rise in rCBF that was observed during the first rCBF measurement. This hypothesis is supported by a previous study, showing that nimodipine-induced hypotension may offset the increase in rCBF (Mohamed et al., 1984).

The present results demonstrate that the phenylalkylamine, (S)-emopamil, can attenuate the acute reduction of rCBF observed immediately after traumatic brain injury. Several experimental studies have reported a beneficial effect of (S)-emopamil on improvement of post-ischemic cerebral blood flow (Bielenberg et al., 1987; Szabo and Hofmann, 1989). However, the mechanism by which (S)-emopamil exerts its effect on rCBF remains to be elucidated. Since serotonin has vasoconstrictive properties, the present finding may not be related only to the blockade of L-type calcium channels, but also to its effects on the serotonin-2 receptor (Hofmann et al., 1989). Szabo (1989) reported that (S)-emopamil increased rCBF in anesthetized rats more effectively than its enantiomer (R)-emopamil. When (S)-emopamil was compared with (R)-emopamil, no differences in calcium-channel-blocking activity were observed, although the affinity of (S)-emopamil for the cerebral serotonin-2 receptor was higher than that of the (R)-enantiomer (Hofmann et al., 1989). It is therefore possible that cerebrovascular effects of (S)-emopamil in the present study may be mediated, at least in part, by serotonin-2 receptors.

Taken together, the results of the present study suggest that the beneficial effect of (S)-emopamil on post-traumatic neurobehavioral and cognitive function observed in our previous investigation (Okiyama et al., 1992) might, in part, be mediated through its effect on rCBF, and that rCBF restoration in acute stage of brain trauma may be an important factor for the prevention of the subsequent pathophysiologic sequelae of traumatic brain injury.

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