Somatosensory Regulation of Regional Hippocampal Blood Flow in Anesthetized Rats

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Abstract The effect of noxious or non-noxious mechanical stimulation of various cutaneous areas on cerebral blood flow in hippocampus was examined with laser Doppler flowmetry in urethane-anesthetized artificially-ventilated rats. Noxious mechanical stimulation (pinching) of the skin on the face, forepaw, chest, or hindpaw for 20 s increased regional hippocampal blood flow (Hpc-BF) and systemic blood pressure, but non-noxious mechanical stimulation (brushing) had no such effect. After the spinal cord was transected at T1 level a forepaw pinch caused no change in blood pressure but still increased Hpc-BF. This suggests that cutaneous noxious stimulation can induce pressor-independent increases in Hpc-BF. The increase in Hpc-BF induced by a forepaw pinch in T1-transected rats was partially reduced by intravenous administration of mecamylamine (2 mg/kg), a nicotinic cholinergic receptor antagonist. Atropine (0.5 mg/kg), a muscarinic cholinergic antagonist was ineffective. These data indicate that the cholinergic vasodilative system is involved in the somatically-induced increase in Hpc-BF via activation of the nicotinic cholinergic receptors.

Key words: cutaneous stimulation, regional hippocampal blood flow, rat, spinal transection.

Somatosensory stimulation can produce reflex changes in various visceral variables in anesthetized animals in whom input of environmental factors which contribute to emotions have been eliminated [1]. These reflex responses vary depending on the sensory modality (e.g., noxious or non-noxious) and on the spinal segmental levels of the somatosensory nerves stimulated. Recently, cutaneous

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sensory stimuli have been shown to affect brain cortical blood flow (cortical BF) in anesthetized rats [2]. In that study, pinching the skin of the forepaw increased cortical BF independent of changes in systemic blood pressure (BP) [2]. Furthermore, it has been shown that somatic afferent stimulation activates neurons in the basal forebrain whose axonal branches project to the cortex [3] and increases the extracellular level of cortical acetylcholine (ACh) [4], and also that activation of the cholinergic neuron originating in the basal forebrain can increase both cortical BF [5] as well as cortical extracellular ACh release [6]. On the basis of these facts, it was suggested that central cholinergic nerve fibers originating in the basal forebrain participate in the somatically-induced increase in regional cortical BF [4]. In parallel with the cortex, the hippocampus also receives cholinergic axonal projections from nerve cells originating in the basal forebrain, especially from the septal complex (i.e., the medial septal nucleus and the nucleus of the diagonal band of Broca) [7, 8].

In alliance with these findings, Dutar et al. [9] found that septo-hippocampal neurons were excited by peripheral noxious somatic stimulation in anesthetized rats, and Cao et al. [10] reported that activation of neurons in the septal complex increased both ACh release and BF in the ventral hippocampus in anesthetized rats. However, Dahlgren et al. [11] reported that non-noxious mechanical stimulation of nasal tissue did not influence regional hippocampal blood flow (Hpc-BF) in anesthetized rats. All these results suggest the idea that noxious cutaneous stimuli increase Hpc-BF via an activation of cholinergic septo-hippocampal neurons. However, this idea has not yet been elucidated.

In the present study, we aimed to examine the response of Hpc-BF induced by somatosensory stimulation, in special reference to the modality and segmental levels of cutaneous sensory stimulation in anesthetized rats. As noxious cutaneous stimuli alters systemic BP, Hpc-BF may be altered secondarily depending on such a change in systemic BP. Therefore, in the present experiments, Hpc-BF was measured both in animals whose spinal cord was intact and also in animals whose spinal cord had been transected at the first thoracic (T1) level as described by Adachi et al. [2] to eliminate any changes in systemic BP following noxious cutaneous stimulation. Finally, the possibility that cholinergic receptors modulate the change in Hpc-BF induced by noxious cutaneous stimulation was examined.

MATERIALS AND METHODS

Twenty adult male Wistar rats weighing 220-450 g were anesthetized with urethane (initial dose 1.1 g/kg, i.p., with supplements of 0.2-0.3 g/kg given through a femoral vein catheter as required), paralyzed with gallamine triethiodide (initial dose 20 mg/kg i.v., plus supplements as required), and artificially ventilated (Model 683, Harvard, U.S.A.) through a tracheal cannula. Precautions were taken to maintain an end-tidal CO₂ of about 4% and an end-tidal O₂ of 16-17%. End-tidal gas concentrations were continuously monitored by a combined CO₂-O₂ gas analy-
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er (1H26, NEC San-ei, Tokyo). Rectal temperature was monitored and automatically maintained at 37.0–38.0°C with a DC heating pad and an infrared lamp (ATB 1100, Nihon Kohden, Tokyo).

Systemic arterial BP was continuously recorded from a femoral artery cannulated with a polyethylene catheter. In 12 rats, the spinal cord was transected at the T1 level. Four percent Ficoll 70 (Pharmacia Fine Chemicals AB, Sweden) in saline solution was intravenously infused when necessary to maintain systolic BP above 70 mmHg.

The Hpc-BF was measured by laser Doppler flowmetry as previously reported [5, 10, 12]. With its body prone, the rat’s head was fixed in a stereotaxic instrument (SR-5, Narishige, Tokyo). After craniectomy, a recording probe (0.8 mm outer diameter) of a laser Doppler flowmeter (LDF; ALF 2100, Advance Co., Ltd., Tokyo) was inserted into the left ventral hippocampus to measure the Hpc-BF about 1 mm below the tip of the probe. The stereotaxic coordinates used were about 5.2 mm posterior to the bregma, about 5.0 mm lateral to the midline and about 5.0 mm directly under the bregma: B −5.2, L 5.0, V 5.0, according to Paxinos and Watson’s atlas [13]. In one rat, the probe was also inserted into the left dorsal hippocampus (B −3.1, L 2.3, V 2.8) and then into the left subiculum (B −7, L 4.5, V 5.0). We selected these three hippocampal areas, whose BF have been found to increase following stimulation of the septal complex [10]. Hpc-BF, expressed in mV from the LDF, was continuously recorded on a polygraph (RM-6000, Nihon Kohden, Tokyo).

The mechanical cutaneous stimuli were delivered to the skin of the face, a forepaw or forelimb, the chest, and a hindpaw or hindlimb for 20 s. The stimuli were delivered either on the left side or on the right side of these skin areas. Non-noxious stimuli were delivered by gently brushing about 4 cm² of skin with a toothbrush at about 2 cm/s and 1 Hz. Noxious stimuli were delivered by pinching about 0.4–0.5 cm² skin with a Péan’s curved hemostatic forceps. The forceps were closed over 2 of the 3 metal teeth. This delivered a force of approximately 3 kg. Both non-noxious and noxious stimuli lasted for 20 s.

In 6 T1-transected rats, the effects of cholinergic receptor antagonists were examined. The following drugs were given intravenously for the stated purposes: atropine (ATR), 0.5 mg/kg (atropine sulfate, Tanabe Pharmaceutical Co., Ltd., Osaka) to antagonize binding muscarinic receptors (5 trials in 5 rats; one trial in each rat); mecamylamine (MEC), 2 mg/kg (mecamylamine hydrochloride, Sigma, U.S.A.) to antagonize binding to nicotinic receptors (6 trials in 4 rats; twice tested in 2 rats). These drugs are known to be permeable to the blood-brain barrier [14].

Data are expressed as mean ± SEM and the Hpc-BF and systolic BP data were analyzed with paired or Student’s t-test.
RESULTS

1. Changes in Hpc-BF after cutaneous mechanical stimulation in spinal cord intact rats

Systolic BP measured before the first cutaneous stimuli was 130 ± 6 mmHg and Hpc-BF was 154 ± 10 mV ($n = 8$) in the spinal cord intact rats.

Figures 1A and B show representative recordings of ventral Hpc-BF and BP just before and after brushing and pinching, respectively, in one rat. Figures 1C and D summarize the results from 8 spinal cord intact rats. As shown in Figs. 1A and C, when a hindlimb was brushed for 20 s there was no significant change in either Hpc-BF or BP. In contrast, when a hindpaw was pinched there were marked increases in both Hpc-BF and BP (Figs. 1B and D). Both responses began within 2 s of the onset of stimulation, and reached their maxima in less than 20 s, and then gradually decreased. Both Hpc-BF and BP returned to their respective baseline.

Fig. 1. Hpc-BF in ventral hippocampus and systolic BP responses to brushing (A, C) and pinching (B, D) of the various skin areas of spinal cord intact, anesthetized rats for 20 s. A, B: Representative recordings of hippocampal blood flow (Hpc-BF) and blood pressure (BP) in one rat after a hindlimb was brushed (A) or a hindpaw was pinched (B) for 20 s. Underbars denote the duration of the stimulus. C, D: Summary of Hpc-BF after ipsilateral stimuli (open columns) and after contralateral stimuli (hatched columns), and BP (dotted columns). The maximum responses are expressed as percentages of the respective values of Hpc-BF and BP just before the onset of stimulation. Each column and vertical bar shows a mean ± SEM ($n = 6$). *$p < 0.05$, **$p < 0.01$, significant difference between the prestimulus basal value and the value measured after stimulation (paired $t$-test). There were no significant differences in Hpc-BF after ipsilateral and after contralateral stimulation (Student’s $t$-test).

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values within 80s after the end of the stimulation.

Generally, when the face, a forelimb, the chest or a hindlimb was brushed there were no significant changes in Hpc-BF and BP (Fig. 1C), when these areas were pinched for 20s both Hpc-BF and BP increased significantly (Fig. 1D). The means of the maximum Hpc-BF values measured after ipsilateral pinching of the face, a forepaw, the chest and a hindpaw were 108, 113, 105, and 114%, respectively, of the prestimulation values. The responses induced by stimulation of the forepaw and hindpaw were larger than those induced by stimulation of the face and chest. There were no significant differences in magnitude of Hpc-BF responses between stimulation of the same region on the left side or the right side of the body (Fig. 1D).

2. Changes in Hpc-BF in response to stimulation of a forepaw after spinal transection

After the spinal cord was transected, Hpc-BF in the ventral hippocampus did not change significantly at rest, but the resting BP significantly decreased. The mean resting systolic BP and Hpc-BF were 76±2 mmHg and 139±11 mV, respectively (n =6).

Figure 2 shows representative recordings of Hpc-BF and BP responses to forepaw pinch before (A) and after (B) spinal transection in one rat, and Figs. 2 C and D summarize the responses of 6 rats. After the spinal transection, a forepaw pinch induced only a marginal change in BP, while the magnitude of the increase in Hpc-BF was similar to that before the spinal transection. The time to attain maximal increase of the Hpc-BF response was longer after the spinal transection.

The changes in Hpc-BF in the dorsal hippocampus and in the subiculum following a forepaw pinch were tested in one rat both before and after the spinal transection, and the magnitude of the responses were essentially the same as those in the ventral hippocampus.

3. Effects of cholinergic blockers on Hpc-BF after spinal transection

The effects of intravenous administration of muscarinic and nicotinic cholinergic receptor antagonists on the responses of Hpc-BF induced by pinching a forepaw were examined in 6 rats in which the spinal cord had been transected at the T1 level.

Figure 3 shows representative recordings in one rat (upper traces) and summarized data of Hpc-BF in the ventral hippocampus from 6 rats (lower column). There were no significant differences between the resting Hpc-BF values before and after administration of the drugs. The response to noxious stimulation was not significantly influenced by atropine injection (0.5 mg/kg, i.v.) (Fig. 3A). The response of Hpc-BF to noxious stimulation was significantly attenuated to about 67% of the noxious stimuli induced increase by MEC injection (2 mg/kg, i.v.) within 10 min (Fig. 3B). MEC (2 mg/kg) is known to abolish Hpc-BF increase induced by chemical stimulation of septal complex [10]. The reduced response
Fig. 2. Hpc-BF and systolic BP responses to pinching the forepaw of anesthetized rats before (A, C) and after spinal transection (B, D). A, B: Representative recordings of Hpc-BF and BP (duration of stimulus indicated by underbars) in one rat. C, D: Summary of Hpc-BF (closed circles) and BP (open circles) in 6 rats before (C) and after (D) spinal transection. The data are expressed as percentages of the respective basal values just before the onset of stimulation. The dashed vertical lines and the heavy bar on the abscissa indicate the time during which the forepaw was stimulated. Each dot and vertical bar indicates a mean ± SEM. *p < 0.05, **p < 0.01, significant difference between the prestimulus basal value and the value after stimulation (paired t-test).

returned to the preinjection control level within 20–30 min.

DISCUSSION

We measured regional Hpc-BF continuously by laser Doppler flowmetry and examined the effects of non-noxious (brushing) and noxious (pinching) mechanical stimulation of various skin areas (face, forelimb and paw, chest, hindlimb and paw) in urethane-anesthetized rats. Noxious stimulation, especially of the paw, always increased Hpc-BF. Furthermore, it is noteworthy that the change in Hpc-BF after ipsilateral stimulation was almost identical to that after contralateral stimulation. These changes in Hpc-BF resemble the previously-reported changes in cortical BF [2].

Somatically-induced changes in cerebral blood flow (CBF) in the spinal cord intact, anesthetized animals are usually accompanied by changes in systemic
Fig. 3. Effects of atropine (ATR), a muscarinic cholinergic receptor antagonist (0.5 mg/kg, i.v.) (A) and mecamylamine (MEC), a nicotinic cholinergic receptor antagonist (2 mg/kg, i.v.) (B) on the responses of Hpc-BF after a forepaw was pinched for 20 s in 6 T1-transected anesthetized rats. Upper traces: Sample recordings. Underbars indicate time of pinching stimulation (20 s). Lower columns: Summarized results. A: Responses of Hpc-BF after saline injection (open column) and after injection of ATR (hatched column) (n = 5). B: Responses of Hpc-BF after saline injection (open column) and after injection of MEC (hatched column) (n = 6). Responses are expressed as the percentage of the control responses before saline injection. Each column and vertical bar indicates mean ± SEM. ∗, p < 0.05; significantly different between the responses after saline and drug injection by Student’s t-test.

arterial BP. The effect of noxious somatic stimuli on local CBF and BP in anesthetized cats was studied by Tsubokawa et al. [15]. They observed a long-lasting increase in CBF and a short-lasting rise in BP after sciatic nerve stimulation. Because of this difference in timing, they suggested that the increase in BP was not responsible for the increase in CBF. Sándor et al. [16] measured changes in thalamic blood flow during stimulation of a sciatic nerve in chloralose-anesthetized dogs. They found a pressor-independent decrease in thalamic blood flow after eliminating the changes in BP with a pressure reservoir system. Adachi et al. [2] transected the spinal cord at T1, and thereby eliminated any influence of a pressor response on the cortical BF after a forepaw was pinched. Sensory impulses from the forepaws could be transmitted to the brain, but impulses from the brain could not reach sympathetic preganglionic neurons in the spinal cord below the T1 level. Using the same procedure, we eliminated the influence of the pressor response on the changes in Hpc-BF induced by pinching a forepaw, and could still observe the increases in Hpc-BF by pinching. The present results suggest that the changes in

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Hpc-BF induced by noxious cutaneous stimulation are at least partly pressor-independent, even in spinal cord intact animals.

A recent report by Kurosawa et al. [4] found that extracellular levels of cortical ACh was increased by somatic afferent stimulation. On the basis of previous reports which found that somatic afferent stimulation activates neurons in the basal forebrain [3, 9], and activation of the cholinergic system can increase both cortical BF [5] and cortical extracellular ACh release [6], Kurosawa et al. [4] suggested that cholinergic nerve fibers originating in the basal forebrain participate in the somatically-induced increase in regional cortical BF.

Dudar [17] reported an increase in hippocampal ACh release after stimulation of the medial septum in rabbits, while Dutar et al. [9] reported that noxious cutaneous stimuli increased the discharges of septo-hippocampal neurons. Cao et al. [10] have shown that stimulation of the septal complex increases both Hpc-BF and extracellular ACh release in the hippocampus in rats, and also that nicotinic receptors but not muscarinic receptors, are involved in the increase in Hpc-BF after stimulation of the septal complex. These results outlined above are in general agreement with the present evidence that the response of Hpc-BF to noxious cutaneous stimulation was suppressed by mecamylamine, a nicotinic cholinergic receptor antagonist, but not by atropine, a muscarinic cholinergic receptor antagonist. Together this suggests that cholinergic fibers, originating in the septal complex and projecting to the hippocampus, are involved in the increase in Hpc-BF induced by somatic afferent stimulation via activation of the nicotinic cholinergic receptors.

The possibility exists that accumulation of local metabolites in the hippocampus in response to an increased activity of hippocampal neurons following somatic afferent stimulation may contribute to the increase in Hpc-BF. However, at this moment, no data is available to confirm this possibility. To clarify the putative contribution of the metabolites to this mechanism, further study is required.

In conclusion, Hpc-BF increased after noxious mechanical cutaneous stimulation irrespective of changes in systemic arterial BP, and this somatically-induced increase in Hpc-BF was partially reduced by intravenous injection of a nicotinic cholinergic receptor antagonist (mecamylamine). This increase in Hpc-BF appears to involve activation of the septo-hippocampal cholinergic system. However, the present increase in Hpc-BF can also be resulted by activation of other cholinergic neurons originating in the sphenopalatine ganglia [18] or in the intrinsic hippocampal neurons [19].

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