Reappraisal of the Corticothalamic and Thalamocortical Interactions that Contribute to the Augmenting Response in the Rat

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Abstract In urethane-anesthetized rats, low frequency electrical stimulation of the thalamic radiation (TR) evoked an augmenting response in the somatosensory cortex (SCx) which was followed by rhythmic slow waves. The augmenting response mainly consists of the incremental secondary response (II-response). Simultaneously, augmentation also occurs in the ventrobasal nucleus of thalamus (VB) on the late component responses, C- and D-waves, to TR stimulation. The latencies of these augmented responses were shorter for the C-wave and the accompanying unit discharges in the VB relay neurons than for the D-wave and the II-response. We hypothesized that the thalamo-cortico-thalamic reverberating circuit was crucial in generating the augmenting response in the SCx. To test this hypothesis, an attempt was made to block temporarily the corticothalamic glutamatergic transmission by means of microinjections of kynurenate (KYN), an antagonist of glutamate, into the VB with a dose of more than 2 mM. This local procedure blocked all of the augmenting phenomena completely with a full recovery after the duration that depended on the dose of KYN. Besides, in the stage of complete blocking of the II-response to the test TR stimuli, the augmentation was able to be restored by adding a short train of high frequency TR stimuli that mimicked a burst discharge of VB relay neurons. These results in support of the hypothesis would reappraise the functional significance of the reverberating circuit in augmentation that has recently been controversial.

Key words: augmenting response, thalamo-cortico-thalamic reverberating circuit, somatosensory cortex, ventrobasal thalamic nucleus, corticothalamic glutamatergic transmission.

It has been well established that low frequency repetitive stimulation of the specific thalamic nucleus induces an augmenting response in the correlated cortical

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area [1,2]. The augmenting response consists of a late incremental potential following a primary response evoked by the thalamocortical afferents. It has been controversial as to whether the interaction of reverberation between the thalamocortical and the corticothalamic pathways is essential in developing augmentation. Recent reports have attributed augmentation solely to the intrinsic cortical organization [3] or to the intracortical axon collaterals of the corticothalamic neurons [4]. In these studies, the augmenting response could be elicited even after the electrolytic or kainic acid lesion of the thalamus, but with some modifications such as decreases in responsiveness of unit firing, or as increases of the threshold and a relatively small maximal amplitude of the response.

The preceding paper [5] has shown that the corticothalamic inputs give rise to powerful facilitation in the VB neurons. Since the transmitter of the corticothalamic pathway could be glutamate [6], the present study was aimed to clarify the contribution of the thalamo-cortico-thalamic circuit to the augmenting response by means of local microinjections of a glutamate antagonist, kynurenate (KYN) [7], into the VB.

MATERIALS AND METHODS

The methods were essentially the same as those described in the preceding paper [5]. Eight rats were anesthetized with urethane (1.5 mg/kg, supplemented with 0.2 mg/kg if needed), while their body temperatures were kept at $36 \pm 1^\circ\text{C}$.

A stereotaxically oriented thalamic radiation (TR) was stimulated to evoke responses in the somatosensory cortex (SCx) and in the thalamic ventrobasal nucleus (VB). Both the responses showed augmentation when elicited by low frequency repetitive stimulation (see below). Kynurenate (KYN) dissolved in physiological saline was applied through a stainless-steel guide cannula (9.0 mm in length, 0.64 mm in outer diameter, and 0.40 mm in inner diameter) that was implanted at a site of the VB: *A*, 5.5–6.0; *L*, 2.5–3.0; and *H*, 4.0–5.0 [8]. A monopolar stainless-steel electrode coated with enamel except for the tip, protruded 0.5–0.7 mm from the tip of the guide cannula was used to record the field potential in the VB. A stainless-steel injection cannula (15.0 mm in length and 0.35 mm in outer diameter) was connected to a 5 μl Hamilton syringe, and was carefully inserted through the guide cannula to the VB. The tip of the injection cannula reached that of the guide cannula, and was kept in place throughout the experiment. Physiological saline or drug solutions of 0.25 μl were injected at a speed of 0.1 $\mu\text{l}/\text{min}$. The sites of the injection cannula and the recording and stimulating electrodes were histologically verified after each experiment.

RESULTS

Augmenting responses of the VB and the SCx

Stimulation of the TR induced positive-negative primary and secondary re-

sponses in the SCx (termed as I- and II-responses, respectively, in Fig. 1C) and three initial negative waves (T1-, T2-, and C-waves cf. [5]) followed by a slow positive wave in the VB. In Fig. 1A, the C-wave occupies most of the early VB response with almost masked T1- and T2-waves. In the preceding paper [5], it had been postulated that the C-wave is produced by the corticothalamic input to the VB. By repetitive TR stimulation of 10 Hz, the C-wave is progressively potentiated (Fig. 1A). Simultaneously recorded discharges of VB relay neuron are progressively increased in parallel with the increasing slow negative wave (Fig. 1B) or with the C-wave (Fig. 1A). Another negative wave following the C-wave, named as D-wave in Fig. 1A, occurs only after the second and subsequent stimuli. Rhythmical oscillatory waves appear after the cessation of stimulation (Fig. 1A).

On the other hand, the simultaneously recorded I-response in the SCx is followed by an augmented II-response during the repetitive TR stimulation (Fig. 1C). The threshold intensity of the TR stimulation with pulses of 50 μ s in width was $18.8 \pm 2.8 \mu$ A for the I-response and $28.4 \pm 1.5 \mu$ A for the augmented II-response (mean $\pm$ SD, $n = 10$). Augmentation of the II-response showed large fluctuation, although the I-response remained almost constant. It should be noted that there is a parallel fluctuation in the II-response of the SCx (Fig. 1C) and the D-wave of the VB (Fig. 1A).

By double TR stimulation, the C-wave in the VB (Fig. 1D-b-d) and the II-response in the SCx (Fig. 1E-b,d) were augmented. The II-response was fluctuated with the D-wave in the VB (Fig. 1D-b,e, E-b,e), as in the case of repetitive stimulation. The peak latencies of the positive and negative component waves were 9.76 ± 0.67 and 16.09 ± 1.45 ms (mean $\pm$ SD, $n = 10$) for the II-response, respectively. The latency of the negative peak of the D-wave was 16.29 ± 0.79 ms ($n = 10$). It seems therefore that both the negative components of the II-response and the D-wave share common mechanisms of generation.

Distribution of I- and II-responses on the SCx surface

The TR-SCx responses are elicited on a restricted region of the cortical surface as shown in Fig. 2. The maximum I-response was observed at 7.5 mm anterior from the lambda suture and 8.0 mm lateral from the midline (Fig. 2A1, B, C). Double shocks were applied to the TR at an interval of 100 ms in order to evoke an augmented II-response. The maximum II-response was observed at 7.0 mm anterior from the lambda suture and 8.0 mm lateral from the midline (Fig. 2A2, B, C). This difference in distribution between the I- and II-responses is small, but may reflect different mechanisms in generating these two responses.

Rhythmic waves and excitability of the SCx after the primary response

The TR stimulation induced long-lasting rhythmic waves both in the SCx (Fig. 3A-a) and in the VB (Fig. 3A-d). There was a time lag in phase between the rhythmic waves in the SCx and those in the VB. When recorded simultaneously (Fig. 3A-c, d), the positive peaks in the VB rhythmic waves roughly corresponded

Fig. 1. Responses in the VB and the SCx evoked by five repetitive stimuli of TR (filled triangle) at 10 Hz. A: TR-VB response, averaged for 10 trials. B: Discharges of a VB relay neuron. C: TR-SCx response, averaged for 10 trials. A, B, and C: simultaneous recording. The records are taken with negativity upward for the field responses and with positivity upward for the unit recording in this and subsequent figures. D and E: Effects of double TR shocks on the VB and the SCx, respectively. a, control first response. The second responses are shown for interval of 10 ms (b), 25 ms (c), or 75 ms (d). Note that the II-responses in the SCx are augmented by double TR shocks at intervals of 10 and 75 ms, but not at an interval of 25 ms.

to the rising phase of the negative SCx wave. When double shocks were applied at various intervals from 25 ms to 1 s, the II-response was markedly augmented during the rising phase of rhythmic SCx waves (Fig. 3A-d) but not during the falling phase (Fig. 3A-c), as illustrated in Fig. 3B (open circles). On the other hand, the C-wave of the TR-VB response was facilitated throughout the period of the rhythmic waves (Fig. 3B, filled circles).

Effects of kynurenate injection into the VB

The experiments of KYN application were conducted on 4 rats. Application

Fig. 2. Distribution of the I- and II-responses on the SCx surface. A1, antero-posterior distribution of the I-response (average for 10 trials). A2, antero-posterior distribution of the II-response (average for 10 trials) to the second TR stimulus at an interval of 100 ms. Traces a, 6.0; b, 6.5; c, 7.0; d, 7.5; e, 8.0 mm anterior from the lambda suture while the lateral distance was fixed at 8.0 mm from the midline. B: Distribution illustrated in A1 and A2. C: Lateral distance measured lateral distribution from the midline while the anterior distance was fixed at 7.0 mm from the lambda suture. The I-responses are indicated with filled circles and the II-responses with open circles.

of KYN into the VB inhibited the C-waves of the TR-VB response allowing the T1- and T2-waves to be left (Fig. 4B, D) and the II-responses in the SCx elicited by double TR shocks without affecting the I-response (Fig. 4A, C). This suppressive action of KYN was reversible (Fig. 4G, H) and the recovery of the II-response was parallel to that of the C-wave (Fig. 4E, F). In 15 min after the injection of 20 mM KYN, the rhythmic waves only in the VB were abolished (Fig. 4D-c) and those in the SCx were decreased (Fig. 4C-c). The rhythmic waves in both regions were abolished by injection of a higher concentration (50 mM) of KYN with recovery earlier in the SCx than in the VB. Early after the injection of KYN and on the way

Fig. 3. Rhythmic slow waves and facilitation of response. A: Sample records (average for 10 trials). a, the TR-SCx response followed by rhythmic slow waves. b and c, the SCx responses to double TR stimulation at intervals of 120 and 200 ms, respectively. d, the VB response at an interval of 200 ms, simultaneous record with c. B: Relative amplitudes (mean $\pm$ SD, $n=10$) of the second II-response (open circles) and the C-wave (filled circles) to their first responses are plotted against the interval of TR double shocks with the same time scale as in A. The amplitude of II-response is measured after subtracting the I-response as illustrated in A-b, and that of the C-wave as in A-d.

of the recovery process, the rhythmic waves in both regions rather advanced the synchronization (Fig. 4E-c, F-c) and gradually returned to the control level in the recovery (Fig. 4G-c, H-c). It is therefore suggested that the optimal activity of the reverberating circuit for the development of rhythmic waves is lower than that in a normal urethane-anesthetized rat.

In Fig. 5A, the relative amplitudes of the augmented II-response and of the C-wave are plotted as a function of the time after the KYN injections with two doses. It is thus shown that the degree and the duration of inhibition are both dose-dependent. Both the II-response and the C-wave exhibited parallel time courses of inhibition. In another series of KYN injections, as plotted in Fig. 5B, it

Fig. 4. Effects of the KYN microinjection into the VB. A: Control TR-SCx responses: the first (a) and second responses (b) to double TR shocks at an interval of 100ms, and late rhythmic waves (c). B: control TR-VB responses: a-c with the same convention as A. All field responses were averaged for 10 trials. C and D: TR-SCx and TR-VB responses 15 min after KYN (20mM) injection, respectively. C-c trace shows rhythmic waves in SCx and D-c the VB field potential with no rhythmic waves 15 min after KYN injection. E and F: TR-SCx and TR-VB responses 90 min after KYN injection, respectively. G and H: TR-SCx and TR-VB responses 120 min after KYN injection, respectively. Note that both the C-waves in the VB and the II-response in the SCx were recovered with each recovered rhythmic wave. Voltage calibrations, 1.0mV applies to TR-SCx responses in a and b; 0.5mV to TR-VB responses in a and b; 0.2mV to TR-SCx responses in c; and 0.1mV to TR-VB responses in c. Time scale of 5 ms applies to a and b; 100ms to c.

was shown that the amplitude of the augmented C-wave was inhibited by KYN in a dose-dependent manner.

Short train TR stimulation was used to mimic the short burst discharges of the VB relay neurons in order to test whether these stimuli added after the double shocks could restore the augmented II-response under the full effect of the KYN inhibition. In Fig. 6, it is demonstrated that the augmented II-response to the second shock (AII-a) is inhibited by KYN (AII-b) but is restored by adding the

Fig. 5. KYN effects on the II-response and C-wave. A: The time course of inhibition in the relative amplitude (mean, $n=10$) of the second II-response to the first II-response elicited by double TR shocks at an interval of 100ms (circles), and of the C-wave expressed with the same conventions (triangle). Two doses of KYN, 10 and 20 mM in concentration, are applied into the VB, as indicated by filled and open symbols, respectively. B: The dose-response curve expressed with the amplitude (mean $\pm$ SD, $n=10$) of the C-wave at 15 min after KYN application. Abscissa, dose in mM. Cont., the C-wave prior to the injection. NaCl, the C-wave 15 min following the saline injection. The records in A and B were acquired from different rats.

high frequency three-pulse stimuli (AII-c). This augmenting effect was not observed on the first II-response (Fig. 6AI-a-c). The augmentation of train stimuli on the second II-response was increased, depending on the number of train pulses

Fig. 6. The augmented II-response depressed by KYN and recovered by high frequency train stimuli that mimic the burst discharges of VB neurons. **A:** Sample records averaged for 10 trials of the first (AI) and the second (AII) TR-SCx responses to the double shocks at a 100 ms interval. **a**, control responses prior to the injection. **b**, the responses following the injection KYN (15 mM). The augmented II-response is abolished. **c**, the responses to the additional train stimuli (300 Hz) following each of the double shocks under the inhibitory action by KYN. **d**, shows the used pattern of TR stimulation. **d1**, double stimuli separated by 100 ms. **d2**, double stimuli followed by additional train stimuli. **B:** Plottings of the amplitude of II-responses versus the pulse number in the train stimuli. **C:** Plottings of the II-response amplitude versus the interval between the test and train shocks (see A). **D:** The II-response amplitude plotted against the intensity of the train stimuli of three pulses. In D, the controls of the first and the second II-responses prior to KYN injection are given to the right of the graph. The intensities of pulses are given by multiples of T that is equal to the intensity of the double shocks used. Each amplitude in B, C, and D was expressed with mean $\pm$ SD ($n = 10$).

(Fig. 6B), the interval between the second test shock and the train stimuli (Fig. 6C), and the intensity of pulses (Fig. 6D).

DISCUSSION

The TR stimulation activates both the corticothalamic and thalamocortical fibers. Augmentation to the double TR stimuli occurred in the II-response in the SCx and the C- and D-waves in the VB. The augmented II-response and the D-wave exhibited function in phase with the rhythmic waves (Fig. 3). The latencies of the augmented responses to the second of double stimuli were shorter for the C-wave and the accompanying discharges in the VB than for the D-wave and the II-response (Figs. 1 and 2).

On the basis of these observations we can set forth a hypothesis on the sequence of the augmenting phenomena in the SCx and VB as follows: The short burst discharges of VB neurons are crucial to induce augmentation of the II-response via the thalamocortical fibers, and the induced excitation of the SCx expressed by the II-response and rhythmic waves in turn facilitate the VB neurons to augment the C- and D-waves via the corticothalamic fibers. This hypothesis thus postulates the active role of the thalamo-cortico-thalamic reverberating circuit.

Evidence in support of this hypothesis has been obtained by KYN injection experiments. First, the KYN injection into the VB blocked augmentation in the II-response completely (Figs. 4 and 5). KYN could act to block the corticothalamic glutamatergic transmission that was critical in augmentation. Second, under the KYN block of the corticothalamic transmissions we stimulate the TR with a few high frequency pulses to mimic the short burst discharges of VB neurons. This procedure was able to restore the II-response to an augmented form (Fig. 6). This finding may further indicate that the corticothalamic input to the VB plays a crucial role in augmentation. Thus, the reverberating circuit between the SCx and the VB should be maintained in action for producing augmentation in both the regions.

In the cat visual cortex, the augmenting response could be evoked by the collateral activation of the corticothalamic neurons even after the thalamic nucleus was chronically lesioned by kainate injections [4]. It is, however, noted that the threshold intensity of the stimulus inducing the augmenting response after the kainate lesion rose from 12 to 400 μ A with a pulse duration of 0.2 ms. Besides, the response to an optimal stimulation fell in amplitude to less than one-eighth of the control before the lesion. The reduction of the response was in part attributed to the functional impairment of the corticothalamic neurons during the chronic process of the experiment. The electrolytic lesion of the VB in the cat also spared the augmenting response but only with reduced discharge probability of SCx neurons to a half and with reduced rate of augmentation to a half [3].

In the present experiment, the TR stimulation activates both the thalamocortical and corticothalamic axons, and thereby activates as well the intracortical

collateral axons of the corticothalamic neurons, both before and after the KYN application into the VB. Yet, there was a complete block of augmentation. The only difference between before and after the KYN application is the block in the trans-thalamic cortical input. This input, when mimicked by the train pulses or after the recovery of KYN effects, was able to restore the augmentation as stated above. Our evidence would be strong enough to reappraise the functional significance of the reverberating circuit in augmenting the SCx and VB responses.

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