Inhibitory modulation of cat somatosensory cortex: a pharmacological study

SIMON BRAILOWSKY1 and ROBERT T. KNIGHT

Department of Neurology, University of California, Davis VA Medical Center, Martinez, CA 94553 (U.S.A.)

(Accepted July 5th, 1984)

Key words: cerebral cortex — somatosensory evoked potentials — pharmacological mapping — GABA — taurine — cat

In anesthetized preparations, GABA and taurine produced rapid, reversible inhibition of the negative component (N20) of the primary somatosensory evoked potential (SEP) without effect on the earlier positivity (P11). This effect was also produced by low doses of 4-aminopyridine. Neither bicuculline or picrotoxin antagonized these drug effects. A predominance of type B GABA receptors in the superficial layers of the somatosensory cortex is proposed.

Neuropharmacological studies of the cerebral cortex have frequently employed excitatory substances such as strychnine, pentylenetetrazol, picrotoxin, bicuculline, and penicillin. Initially, their effects were interpreted as direct increases in excitatory mechanisms. Currently, however, the excitatory action of these drugs are felt to be secondary to blockade of inhibitory mechanisms.

The most widely studied inhibitory amino acid candidates in the cerebral cortex are gamma-aminobutyric acid (GABA) and taurine. The contribution of these inhibitory agents to the generation of intracortical evoked activity in the primary sensory regions is not well-defined. This report describes the actions of these inhibitory agents on the somatosensory evoked potential (SEP) recorded from the primary somatosensory area (SI) of the cat cortex.

Experiments were performed on 14 adult, female cats (2.5–3.5 kg) under sodium pentobarbital anesthesia (initial dose = 35 mg/kg i.p.). The animals were artificially ventilated with continuous monitoring of the EKG and the expired CO2 concentration (< 4%). An IV line was maintained through which the barbiturate was delivered continuously at slow rate. Anesthetic concentration was maintained at levels which suppressed background EEG activity. Care was taken to maintain body temperature (37°C) throughout the experiment. The animals were placed in a stereotaxic frame and a bilateral craniotomy was performed. Somatosensory evoked potentials (SEPs) were recorded to square wave pulses of 0.15 ms duration and 1–10 mA intensity, delivered through a stimulus isolation unit at 2 Hz to the contralateral forepaw pad. The stimulus intensity was adjusted to produce a slight twitch of the forepaw. Recordings were made with small (< 1 mm) silver chloride balls. All recordings were referenced to a frontal sinus screw. A ground electrode was inserted into the neck muscles. The signals were amplified using a 3–3000 Hz band pass and fed into a 4-channel general purpose signal averager (Nic-1172). Continuous monitoring of the EEG was obtained. For the averaged primary evoked response, 32 epochs of 60 ms each were averaged with a 10-ms pre-stimulus baseline. Clear SEPs to individual stimuli were seen in the suppressed EEG. Differences in waveforms formed by digital subtraction of the drug-treated SEP from the control SEP were used for analysis of the pharmacological effects.

In each animal, the SI projection area was mapped through the intact dura for maximum amplitude and definition of the primary cortical SEP. The dura was

---

1 On leave of absence from the Dept. of Pharmacology, Faculty of Medicine, National University of Mexico.

Correspondence: R. T. Knight, Dept. of Neurology, Univ. of Calif., Davis, V.A.M.C., 150 Muir Road, Martinez, CA. 94553, U.S.A.

0006-8993/84/$03.00 © 1984 Elsevier Science Publishers B.V.
then cut and the cortex exposed. The cortex was bathed throughout the experiment with warmed Ringers lactate solution (37 °C). This solution was also used to wash the cortex after each drug application. Drugs (see Table I) were freshly prepared and administered diluted in saline at 37 °C through a microsyringe. Each drug application employed a total volume of 1 μl. All solutions, with the exception of bicuculline, were adjusted to pH 7.4.

Recordings of the evoked responses were obtained immediately before GABA, taurine or 4-AP application and at 5 s, 30 s, 1 min, and 5 min thereafter.

**Table I**

<table>
<thead>
<tr>
<th>Drugs and doses used (in μg/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibitory transmitters</strong></td>
</tr>
<tr>
<td>GABA</td>
</tr>
<tr>
<td>Taurine</td>
</tr>
<tr>
<td><strong>Antagonists</strong></td>
</tr>
<tr>
<td>Bicuculline</td>
</tr>
<tr>
<td>Picrotoxin</td>
</tr>
<tr>
<td><strong>Transmitter releaser</strong></td>
</tr>
<tr>
<td>4-aminopyridine</td>
</tr>
</tbody>
</table>

Note: all drugs purchased from Sigma (St. Louis, MO).

---

**Fig. 1.** Topography of the Somatosensory Evoked Potential (SEP) in the cat to contralateral forepaw stimulation. Note the enhanced amplitude of the negative (downwards) component (N20) in the vicinity of the coronal gyrus. The horizontal line across the figure indicates the stereotaxic zero plane. Each tracing is the sum of 32 stimuli.
The affected area was washed for 10 s with warmed (37 °C) Ringer lactate after the first post-drug average was completed. After replication, either bicuculline or picrotoxin was applied 5 to 60 s prior to GABA or taurine application and the averaging and washing scenario was repeated. Drug effects were considered to be irreversible when recordings showed no return to baseline conditions over a 60-min period. At this time, recording was switched to the homologous contralateral area and the above procedures repeated.

At the end of the experiment, an overdose of barbiturate was given.

Mapping of evoked activity. The primary somatosensory evoked potential (SEP) consisted of an initial positivity (P11) at 10.9 ± 1.8 ms latency (range = 8.4–13.8) followed by a more variable negativity (N20) at 19.8 ± 3.3 ms latency (range = 15–25.8 ms). Similar latencies have been reported by others. The SEP was found to have the largest amplitude and clearest component definition in the region of the posterior bank of the coronal sulcus (See Fig. 1).

Striking differences were found when moving the electrode across this region. Both decreases and inversions of the SEP components were observed. The variability of the SEP components on repeated averages was minimal, except when the anesthesia level diminished and corresponding background EEG increased or when the cortical temperature changed (as when washed with Ringer lactate above 37 °C). Increasing temperature routinely produced voltage increase in all SEP components. In those regions in which the N20 was not well-developed, the drug effects to be discussed were minimal or not apparent.

Effect of drugs. GABA and taurine induced consistent changes in the SEP and both substances exhibited similar kinetics and actions at doses above 10 μg/μl (for dosages, see Table 1). The main effect consisted in a marked decrease or abolition of the N20 component of the SEP in all animals examined (P < 0.001, see Fig. 2). The onset latency of drug effects on the N20 component was rapid, with changes apparent on the SEP to the first stimulus following drug application. Washing the cortex for 10 s returned the SEP to baseline conditions within two min. The recovery period of both the GABA and taurine effects was prolonged by increasing the dose or delaying the washing of the cortex.

![Fig. 2. Effects of topical application of GABA (10 μg in 1 μl) on cat's somatosensory cortex (open star). SEPs amplified from the squared area in the diagrammed cat brain. Note that no changes were detected in the SEPs recorded outside the application site (asterisk, cross).](image)

The effects on the N20 component showed a plateau at GABA doses beyond 50 μg/μl. In addition, the drug effects on the N20 were restricted to a small region of cortex with no changes apparent in SEPs recorded at distances as close as 3–4 mm from the application site (see Fig. 2).

Differences in waveforms revealed that GABA administration began to remove the negativity at 11 ms post-stimulus with a peak effect at about 20 ms, corresponding to the peak latency of the N20 component. Similar effects and kinetics were observed with taurine (see Figs. 3 and 4). 4-Aminopyridine (4-AP), a K⁺-channel blocker, when administered at low doses (1 μg/μl) had effects similar to those of GABA or taurine. Higher doses (above 3.3 μg/μl) produced an irreversible, high amplitude paroxysmal EEG, with a resultant marked degradation of the SEP signal to noise ratio.

In a few animals, the P11 component was also affected, showing slight amplitude increases. These were most prominent when washing of the cortex was delayed past 45 s after drug administration.

Neither bicuculline or picrotoxin had any protective effect on the N20 component when applied at non-epileptogenic doses (under 3 and 12.5 μg/μl, respectively) from 5 to 60 s before GABA or taurine challenge. Higher concentrations of these convulsants produced irreversible paroxysmal epileptogenic patterns preventing further averaging.

This study describes the topography and time course of GABA, taurine and 4-AP effects on the
primary cortical somatosensory evoked potential. These drugs produced a rapid abolition of the prominent negative component (N20) of the SEP with little or no effect on the initial positive component (P11) of the SEP. The inconsistency of drug effects on the surface recorded P11 component, felt to be generated by current sinks in depolarized cell bodies in the cortical depth\textsuperscript{5,17}, coupled with the rapid, local and reversible actions of GABA and taurine on the N20 component is in accord with the suggested superficial cortical origin of the negativity of the primary cortical response. This negative component of the primary cortical evoked response has been proposed to be generated by depolarization of apical dendrites of pyramidal cells due either to axo-dendritic collaterals or to ascending depolarization of the dendritic tree\textsuperscript{5,11,16,17}. A similar susceptibility to both GABA and taurine has been reported for the negative component of the direct cortical response (CDR)\textsuperscript{12,16}, which is also considered to originate from dendritic activities in the uppermost cortical layers.

Although the findings of rapid, reversible effects on N20 by surface drug application supports a superficial neuronal source, drug-induced effects on superficial neuronal elements with secondary changes on deep cortical generators cannot be ruled out with this data. Further analysis of the drug effects employing current source density and multunit techniques may help to resolve this issue.

Since 4-AP, a drug that increases neurotransmitter release non-specifically\textsuperscript{22}, also produced a rapid inhibition of the N20 component, inhibitory processes may be a predominant phenomenon in the superficial layers of the cortex. Indeed, the synthesizing enzyme for GABA, glutamic acid decarboxylase (GAD), is widely distributed in these areas\textsuperscript{8}.

Endogenous GABAergic interneurons are likely to be involved in the effects that we describ-

---

**Fig. 3.** GABA effects on SEP. Averaged potentials and difference waves (i.e. control SEP subtracted from drug condition SEP) at 5, 30 and 60 s after drug application. Note that the drug effects are seen predominantly in the latency range of the negative component of the SEP. Each tracing is the sum of 32 stimuli.
Fig. 4. Taurine effects on cat's SEP. Same format as Fig. 3. Note the similarity with the GABA effects.

e1-4,6,10,11,18. The cellular candidates would be Golgi type II cells, small basket cells, and chandelier cells3,9,21 all of which are found in layers II and III. These GABAergic interneurons may function through inhibitory axo-dendritic influence on the apical dendritic arborizations of primary sensory region neurons and in this way would be capable of modulating cortical excitability at an early stage of sensory processing15.

We were unable to antagonize the GABA effects with bicuculline or picrotoxin supporting Woodbury's statement about the relative insensitivity of the cortex to these drugs23. In the case of bicuculline, this may be due to a predominance of GABA type B receptors at the cortical level, which are insensitive to the drug20.

An inhibitory action of taurine on the negative component (N20) of the SEP was routinely recorded. Using microiontophoresis and/or direct cortical stimulation (CDR), taurine has been shown to produce inhibition in the cortex12,14,19. However, no anatomical data on taurinergic cortical neurons is available at this time.

The authors would like to thank Dr. K. F. Killam and Dr. D. L. Woods for their critical reading of the manuscript. This research was supported by the Medical Research Service of the Veterans Administration and by a grant from the NIA (AG 02484).


