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Excitatory responses evoked in prefrontal cortex by mediodorsal thalamic nucleus stimulation: influence of anaesthesia

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Abstract

The prefrontal cortex and the mediodorsal thalamic nucleus are reciprocally connected through excitatory amino acid pathways. Cortical excitatory responses resulting from activation of either the mediodorsal thalamic nucleus-prefrontal cortex pathway (short latency) or the recurrent collaterals of prefrontal cortex-mediodorsal thalamic nucleus neurons (long latency) can be discriminated mainly by their latency. The present study was undertaken to compare the effects of halothane and ketamine anaesthesia on these cortical excitatory responses and to establish their pharmacological characteristics using microiontophoretic application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D,L-2-amino-5-phosphonovaleric acid (APV), the specific antagonists of D,L-α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA) and *N*-methyl-D-aspartic acid (NMDA) receptors respectively. The number of prefrontal cortex cells which presented short or long latency excitatory responses was smaller in halothane- than in ketamine-anaesthetized rats. Whatever the anaesthetic used, short latency responses were blocked by CNQX and not affected by APV. Long latency responses were mainly blocked by APV and occasionally by CNQX in halothane-anaesthetized rats, while they were only blocked by CNQX in ketamine-anaesthetized animals. Therefore, halothane seems to preferentially reduce evoked responses mediated by AMPA receptors while ketamine completely abolishes evoked responses involving NMDA receptors. Moreover, the present data confirm that excitatory responses resulting from the activation of the mediodorsal thalamic nucleus-prefrontal cortex pathway are mainly mediated by AMPA receptors. In addition, they demonstrate that cortical responses linked to the activation of recurrent collaterals from prefrontal cortex-mediodorsal thalamic nucleus neurons involve both AMPA and NMDA receptors.

Keywords: Mediodorsal thalamic nucleus; Prefrontal cortex; Anesthesia; Excitatory amino acid

1. Introduction

Anatomical and electrophysiological studies have indicated that corticofugal neurons and their recurrent collaterals as well as the major input to the cerebral cortex which arises from the thalamus use excitatory amino acids, very likely glutamate and/or aspartate, as neurotransmitters (see for review Salt and Herrling, 1991). Such reciprocal excitatory amino acid connections have been demonstrated recently between the prefrontal cortex and the mediodorsal thalamic nucleus in the rat using the method of selective retrograde labeling with D-[^{3}H]aspartate (Streit, 1980). Indeed, retrograde labelled neurons were found bilaterally in deep layers, predominantly layer VI of the prefrontal cortex following D-[^{3}H]aspartate injection into the mediodorsal thalamic nucleus (Ray et al., 1992), and we have observed numerous retrograde labelled cells in the mediodorsal thalamic nucleus following D-[^{3}H]aspartate injections into the prefrontal cortex (Pirot et al., 1994). Furthermore, excitatory responses can be recorded in prefrontal cortex neurons during electrical stimulation of the mediodorsal thalamic nucleus (Canedo, 1982; Ferron et al., 1984; Gigg et al., 1992; Pirot et al., 1994). These responses could result from the activation of both the mediodorsal thalamic nucleus-prefrontal cortex pathway and the recurrent collaterals of antidromically driven corticothalamic neurons. Recently, these two types of excitatory responses were recognized in halothane-anaesthetized rats on the basis of their electrophysiological characteristics (Pirot et al., 1994). Excitatory responses evoked by the activa-

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tion of the mediodorsal thalamic nucleus-prefrontal cortex pathway occur with short latency and are mainly recorded in cells from layer II and III of the ipsilateral prefrontal cortex. They are observed by stimulating the mediodorsal thalamic nucleus at a low frequency (0.3–1 Hz) and disappear at higher frequencies. Excitatory responses evoked by the activation of recurrent collaterals of prefrontal cortex-mediodorsal thalamic nucleus fibers are characterized by their long latency, mainly observed in the deep layers of both the ipsilateral and the contralateral prefrontal cortex and they result from mediodorsal thalamic nucleus stimulation at higher frequencies (3–10 Hz).

General anaesthetics depress excitatory synaptic transmission, in addition to their potentiating effects on inhibitory synapses (Richards, 1980; Franks and Lieb, 1994). Furthermore, some anaesthetics influence glutamatergic transmission by acting directly on D,L-α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA) and/or *N*-methyl-D-aspartic acid (NMDA) receptors. For example, ketamine, a dissociative anaesthetic, is a selective non-competitive inhibitor of NMDA receptors but does not affect AMPA receptors (Thomson et al., 1985; Kemp et al., 1987; Franks and Lieb, 1994). On the other hand, recent experiments in vitro have suggested that halothane, a volatile anaesthetic, decreases AMPA responses but does not affect NMDA responses or does so only slightly (Carlà and Moroni, 1992; Peoples and Weight, 1992).

Therefore, in the present study, attempts were made to compare the effects of halothane and ketamine anaesthesia on excitatory responses evoked in the prefrontal cortex by mediodorsal thalamic nucleus stimulation, and to characterize the subtype(s) of glutamate receptors (AMPA and/or NMDA) involved in these excitatory responses. For this purpose, the effects of iontophoretic application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D,L-2-amino-5-phosphonovaleric acid (APV), the antagonists of AMPA and NMDA receptors respectively, on responses evoked by mediodorsal thalamic nucleus stimulation were compared in halothane- and ketamine-anaesthetized rats. Excitatory responses evoked by the activation of recurrent collaterals of the prefrontal cortex-mediodorsal thalamic nucleus pathway were discriminated from those induced by the activation of mediodorsal thalamic nucleus-prefrontal cortex neurons by their different latency and frequency sensitivity to mediodorsal thalamic nucleus stimulation.

2. Materials and methods

2.1. Animals

Experiments were performed on 38 male Sprague-Dawley rats (Iffa Credo, L'Arbresle, France) weighing 250–300 g. Twenty-four rats were anaesthetized with halothane (0.8–1% in air) delivered intratracheally by a continuous flow pump. Fourteen animals were anaesthetized with ketamine (80 mg/kg i.p.), additional 50 mg/kg i.m. injections being made in order to maintain a stable level of anaesthesia. The depth of anaesthesia was evaluated from the limb withdrawal reflex and was kept just sufficient to produce areflexia. The animals were fixed in a stereotaxic head frame (Horseley Clarke Apparatus, Unimécanique, Epinay-sur-Seine, France). Body temperature was servocontrolled at 37°C with a homeothermic warming blanket (Harvard Bioscience).

2.2. Electrical stimulation

Following trepanation, a bipolar coaxial stimulating electrode (tip–barrel distance, 300 μm; diameter, 200 μm) was positioned into the mediodorsal thalamic nucleus (A: + 6.4 mm; L: + 0.4 mm; H: 4.6 mm) according to the atlas of Paxinos and Watson (1986). The electrical stimulation of the mediodorsal thalamic nucleus consisted of square-wave pulses (0.5 ms duration, 100–500 μA intensity) delivered at frequencies of either 0.1–1 Hz or 3–10 Hz.

2.3. Extracellular recordings

A fine incision was made in the dura at the level of the medial prefrontal cortex in order to safely position the recording electrode glued to the iontophoretic pipette. The activity of prefrontal cortex cells from layers II and VI (A: 11.7–13.2 mm; L: 0.3–1.2 mm; 2–4 mm from the dura) was recorded extracellularly with glass micropipettes filled with 4% Pontamine Sky Blue dissolved in a 0.4 M NaCl solution (impedance, 6–10 MΩ). This activity was amplified with a WPI DAM-5A differential preamplifier (filters, 100 Hz low-frequency and 30 kHz high-frequency) and displayed on a memory oscilloscope. Action potentials were separated from noise using a window discriminator and were fed to a computer (CED 1401 interface connected to an IBM PC) to generate rate or peristimulus histograms.

2.4. Microiontophoresis

Five-barrel glass micropipettes were pulled with a vertical pipette puller (Narishige, Tokyo, Japan) and their tip was broken to a diameter of 1–3 μm. Each five-barrel microelectrode was glued to the recording electrode under microscopic control with a tip–tip distance of 10–20 μm, since this particular disposition reduces current artefacts. A retaining current (+10 to +20 nA) was applied to each barrel to prevent passive diffusion between ejection periods. The effects of iontophoretic currents of at least 50 nA (agonists) and 100

nA (antagonists) were tested before a cell was considered unresponsive.

Individual barrels of the microiontophoretic electrode were filled with one of the following compounds dissolved in a 200 mM NaCl solution: *N*-methyl-D-aspartic acid (NMDA, 50 mM, pH 9), D,L-α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA, 10 mM, pH 8), D,L-2-amino-5-phosphonovaleric acid (APV, 50 mM, pH 9), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 1 mM, pH 8). NMDA and APV were obtained from Sigma (St. Louis, MO, USA), while AMPA and CNQX were from Tocris Neuramin (Buckhurst Hill, UK).

2.5. Data acquisition

Excitatory responses of prefrontal cortex cells evoked by mediodorsal thalamic nucleus stimulation were quantified on peri-stimulus time histograms using 1-ms bins, corresponding to at least 50 cummulative sweeps. These responses were considered to be blocked by either CNQX or APV when they were reduced by at least 50%. Responses of these prefrontal cortex neurons to iontophoretic application of AMPA and NMDA and of their respective antagonists, CNQX and APV, were also tested. For each cell investigated, the mean basal firing rate during a 60-s period preceding the AMPA (5 s) or NMDA (10 s) application was determined. Responses to iontophoretic application of these compounds were quantified on rate histograms using 2-s bins. AMPA- and NMDA-evoked excitations were considered to be blocked by either CNQX or APV when the antagonist decreased the excitatory response by at least 50%.

2.6. Histological verification

At the end of each experiment, the animals were killed by decapitation. The tip of the stimulating electrode was marked by electrical deposit of iron (10 μA anodal current, 15 s) and this point was localized on histological sections following a ferri–ferrocyanide reaction. The location of the tip of the recording electrode was marked by iontophoretic ejection of Pontamine Sky Blue (8 μA cathodal current, 20 min) in order to determine the position of the recorded cells in the prefrontal cortex. The localization of these blue points was observed on serial coronal sections (80 μm) stained with cresyl violet.

3. Results

3.1. Effect of iontophoretic application of AMPA and NMDA on the spontaneous activity of prefrontal cortical cells in halothane- or ketamine-anaesthetized rats

Prefrontal cortex neurons exhibited a broad pattern of spontaneous activity from almost silence (< 0.5 Hz) to 30 spikes/s. In halothane-anaesthetized rats, the majority of these cells were almost silent or presented a very low discharge rate with often irregularly spaced bursts (with 3–5 spikes in a burst). In ketamine-

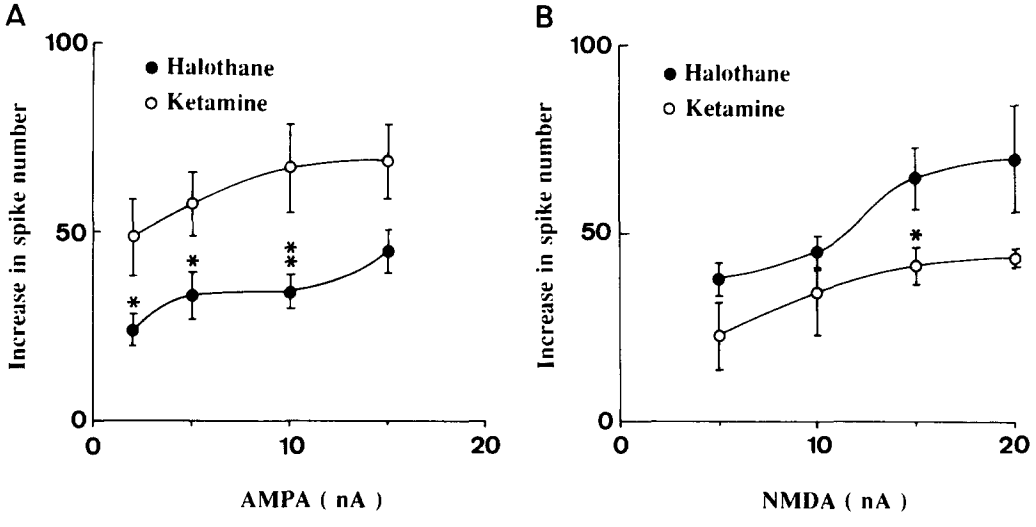

Fig. 1. Current-response curves of the excitatory effects evoked by iontophoretic application of AMPA and NMDA in prefrontal cortex neurons of halothane- and ketamine-anaesthetized rats. Each point represents the mean increase in spike number ± S.E.M. for at least 4 cells. For each cell, the increase in spike number corresponds to the augmentation in firing rate over 10 s after the beginning of AMPA application (5 s) and for 20 s after the beginning of NMDA application (10 s). A: Reduced ability of AMPA to increase the firing rate of prefrontal cortex neurons in halothane-as compared with halothane-anaesthetized rats. B: Reduced ability of NMDA to increase the firing rate of prefrontal cortex neurons in ketamine-as compared with ketamine-anaesthetized rats. The difference between the curves is significant with $P < 0.05$ (Newman-Keuls test following significance according to analysis of variance). * $P < 0.05$; ** $P < 0.01$ halothane- versus ketamine-anaesthetized rats at the same current.

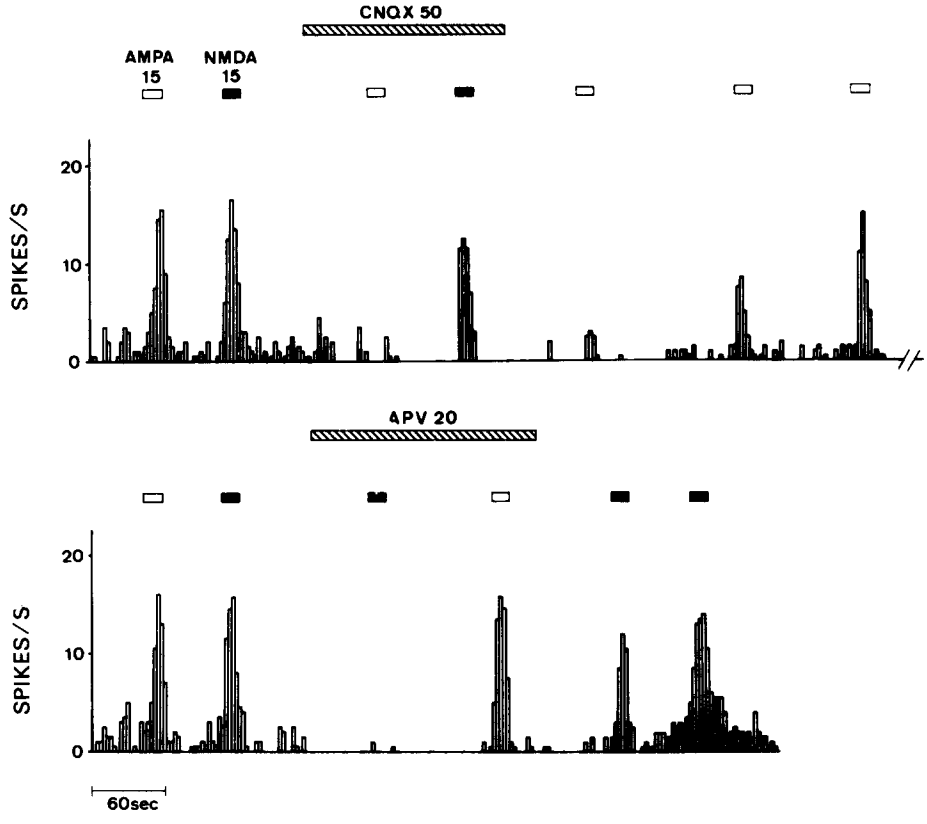

Fig. 2. Cumulative rate histogram illustrating the effects of iontophoretic application of CNQX and APV on AMPA- and NMDA-induced activation of the same prefrontal cortex neuron in a halothane-anaesthetized rat. Both AMPA and NMDA induced a reversible increase in the spontaneous activity of this prefrontal cortex cell. The AMPA-evoked response was blocked by CNQX and not by APV. In contrast, the NMDA-evoked response was blocked by APV but persisted during CNQX application. Note that CNQX and APV applied at currents which suppress the effects of AMPA and NMDA respectively decreased the spontaneous activity of this neuron. Horizontal bars indicate the duration of each iontophoretic application and the numbers above are the ejection currents in nanoamperes.

anaesthetized rats, most of the cells had an irregular firing pattern ranging from 0.5 to 10 Hz, and burts were rarely observed.

Iontophoretic application of AMPA or NMDA induced a reversible and current-dependent increase in the discharge rate of all prefrontal cortex cells tested in both halothane- and ketamine-anaesthetized animals. The excitatory effect of NMDA was characterized by a burst pattern of action potential firing contrasting with AMPA responses which consisted of an increase in, and a more regular pattern of, discharge. Current-response curves of the excitatory effects evoked by the two agonists indicated that prefrontal cortex neurons were significantly ($P < 0.05$, analysis of variance with Newman-Keuls test) less sensitive to AMPA in halothane-($n = 47$) than in ketamine- ($n = 20$) anaesthetized rats and reciprocally slightly less sensitive to NMDA under ketamine ($n = 20$) than halothane ($n = 37$) anaesthesia (Fig. 1).

Iontophoretic application of CNQX (20–60 nA) blocked the AMPA-evoked responses in halothane- and ketamine-anaesthetized rats similarly (90 ± 4% and 98 ± 1% respectively). In some cases, CNQX slightly decreased the NMDA-evoked responses at currents which completely blocked the responses to AMPA. APV (20–80 nA) antagonized in a similar way the NMDA-evoked responses in halothane- and ketamine-anaesthetized rats (89 ± 3% and 84 ± 7% respectively). In none of the prefrontal cortex neurons tested did the application of APV affect the AMPA-evoked excitation.

In addition, when prefrontal cortex cells were active,

Table 1
Short and long latency excitatory responses induced in prefrontal cortex neurons by mediodorsal thalamic nucleus stimulation in halothane- and ketamine-anaesthetized rats

	Number of cells tested	Number of cells excited	Latency (ms)
Short latency excitatory responses			
Halothane	465	66 (13%)	3.5 ± 0.1
Ketamine	391	89 (23%) [a]	3.9 ± 0.1
Long latency excitatory responses			
Halothane	601	95 (16%)	14.7 ± 0.3
Ketamine	278	150 (54%) [b]	16.1 ± 0.2

[a] $P < 0.01$, [b] $P < 0.001$ (χ^2 test) number of excited cells significantly greater in ketamine-anaesthetized rats.

Table 2
Effects of iontophoretic application of CNQX and APV on short and long latency cortical excitatory responses induced by mediodorsal thalamic nucleus stimulation in halothane- and ketamine-anaesthetized rats

	CNQX		APV	
	Number of cells tested	Blockade of excitatory response	Number of cells tested	Blockade of excitatory response
Short latency excitatory responses (mediodorsal thalamic nucleus-prefrontal cortex pathway)				
Halothane	16	14 (88%)	19	1 (5%) [b]
Ketamine	11	10 (91%)	9	0 (0%) [b]
Long latency excitatory responses (collaterals from prefrontal cortex-mediodorsal thalamic nucleus neurons)				
Halothane	19	6 (32%)	17	12 (71%) [a]
Ketamine	65	42 (65%) [c]	34	0 (0%) [b]

[a] $P < 0.02$, [b] $P < 0.001$ (χ^2 test) number of excitatory responses blocked by APV and CNQX, significant differences. [c] $P < 0.001$ (χ^2 test) number of long latency excitatory responses blocked by CNQX significantly greater in ketamine-anaesthetized rats than in halothane-anaesthetized rats.

in most cases, when CNQX or APV were applied at currents which suppressed the effects of their respective agonist, there was a decrease in the spontaneous activity of the cells (Fig. 2). The CNQX- and APV-induced blockade of the spontaneous activity of the cells was 94 ± 7% and 93 ± 5%, respectively, in halothane-

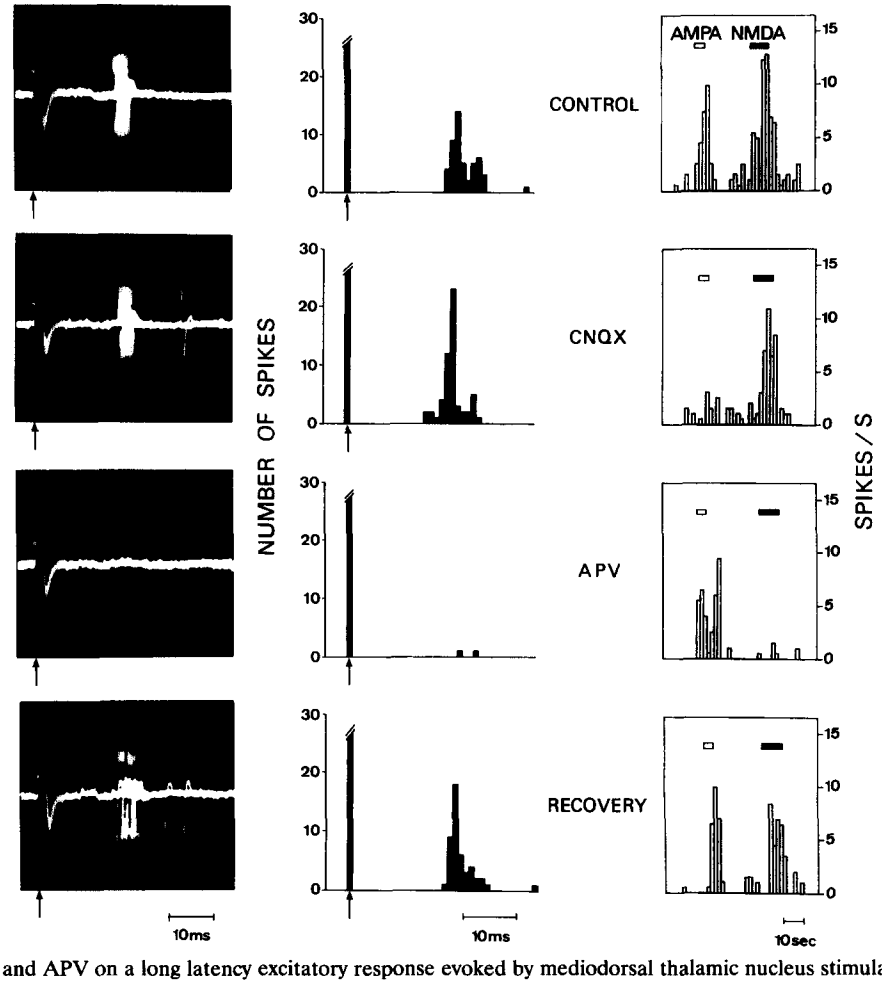

Fig. 3. Effects of CNQX and APV on a long latency excitatory response evoked by mediodorsal thalamic nucleus stimulation and on AMPA- and NMDA-induced activation of the same prefrontal cortex neuron in a halothane-anaesthetized rat. The excitatory response to high frequency stimulation (5 Hz) of the mediodorsal thalamic nucleus is represented by 5 superimposed oscilloscope sweeps (left panel) and by peristimulus histograms (50 sweeps; middle panel). Rate histograms illustrate AMPA- and NMDA-evoked excitations of the same prefrontal cortex neuron (right panel). From top to bottom: responses under control conditions; the application of CNQX (80 nA) did not affect the mediodorsal thalamic nucleus- and NMDA-evoked excitation but blocked the AMPA response; the application of APV (15 nA) completely antagonized the mediodorsal thalamic nucleus- and NMDA-evoked excitations but did not affect the AMPA-evoked response; 2 min after the cessation of APV application, recovery of the excitatory response was observed. Horizontal bars indicate the duration of AMPA (2 nA) and NMDA (7 nA) iontophoretic applications. Arrows indicate the stimulus artefact.

anaesthetized rats and 93 ± 5% and 80 ± 6%, respectively, in ketamine-anaesthetized animals.

3.2. Excitatory responses evoked by mediodorsal thalamic nucleus stimulation in the prefrontal cortex of halothane- or ketamine-anaesthetized rats

The excitatory responses evoked in prefrontal cortex neurons by mediodorsal thalamic nucleus stimulation and resulting from either the activation of the recurrent collaterals from the prefrontal cortex-mediodorsal thalamic nucleus neurons or the activation of the mediodorsal thalamic nucleus-prefrontal cortex pathway were investigated in ketamine- or halothane-anaesthetized rats. Based on previous observations (Pirot et al., 1994), the long latency excitatory responses (> 10 ms) of prefrontal cortex neurons in deep cortical layers (V–VI) evoked by mediodorsal thalamic nucleus stimulation at a frequency of 3–10 Hz were attributed to activation of recurrent collaterals from prefrontal cortex-mediodorsal thalamic nucleus projecting neurons while the short latency responses (< 4 ms) of cells from layer II–III evoked by mediodorsal thalamic nucleus stimulation at a frequency of 0.3–1 Hz were considered to result from activation of mediodorsal thalamic nucleus-prefrontal cortex cells. As shown in Table 1, the proportion of prefrontal cortex cells responding with a long latency to mediodorsal thalamic nucleus stimulation (3–10 Hz) was higher in ketamine- than in halothane-anaesthetized rats and this was also the case for the prefrontal cortex cells presenting short latency responses to mediodorsal thalamic nucleus stimulation (0.3–1 Hz).

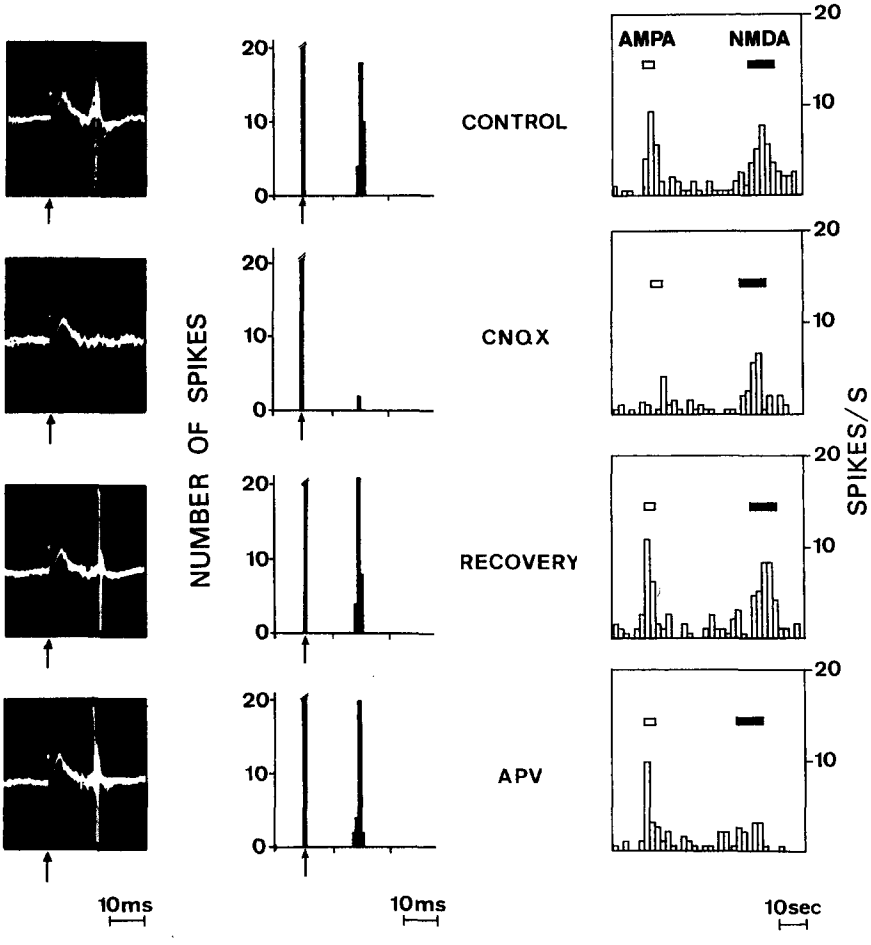

Fig. 4. Effects of CNQX and APV on a long latency excitatory response evoked by mediodorsal thalamic nucleus stimulation and on AMPA- and NMDA-induced activation of the same prefrontal cortex neuron in a ketamine-anaesthetized rat. The excitatory response to high frequency stimulation (5 Hz) of the mediodorsal thalamic nucleus is represented by 5 superimposed oscilloscope sweeps (left panel) and by peristimulus histograms (50 sweeps; middle panel). Rate histograms illustrate AMPA- and NMDA-evoked excitations of the same prefrontal cortex neuron (right panel). From top to bottom: responses under control conditions; the application of CNQX (45 nA) completely antagonized the mediodorsal thalamic nucleus- and AMPA-evoked excitations and had little effect on the NMDA-evoked response; 2 min after the cessation of CNQX application, recovery of the excitatory response was observed; the application of APV (30 nA) did not affect the mediodorsal thalamic nucleus- and AMPA-evoked excitation but blocked the NMDA response. Horizontal bars indicate the duration of AMPA (3 nA) and NMDA (5 nA) iontophoretic applications. Arrows indicate the stimulus artefact.

3.3. Effects of CNQX and APV on the excitatory responses evoked by mediodorsal thalamic nucleus stimulation in the prefrontal cortex of halothane- or ketamine-anaesthetized rats

The effects of iontophoretic application of CNQX and APV on the long and short latency evoked responses were compared in halothane- and ketamine-anaesthetized rats, CNQX and APV being applied at currents which selectively blocked the AMPA- and NMDA-evoked responses respectively.

In halothane-anaesthetized rats, the long latency excitatory responses evoked in prefrontal cortex cells by mediodorsal thalamic nucleus stimulation (3–10 Hz) were preferentially blocked by APV (71% of the cells tested compared with only 32% with CNQX) (Table 2 and Fig. 3). The effects of CNQX and APV were also tested successively in 16 of these cells. Both CNQX and APV blocked the responses in 3 cells, APV but not CNQX inhibited the responses in 9 cells, CNQX but not APV blocked the excitatory responses in only 2 cells, and finally CNQX and APV were ineffective in 2 cells. This indicated that NMDA receptors were involved in most but not all cases. In contrast, in ketamine-anaesthetized rats, the long latency excitatory responses were blocked only by CNQX (65%) and not by APV (Table 2 and Fig. 4).

As previously shown (Pirot et al., 1994), in halothane-anaesthetized rats, the short latency excitatory responses evoked in prefrontal cortex cells by

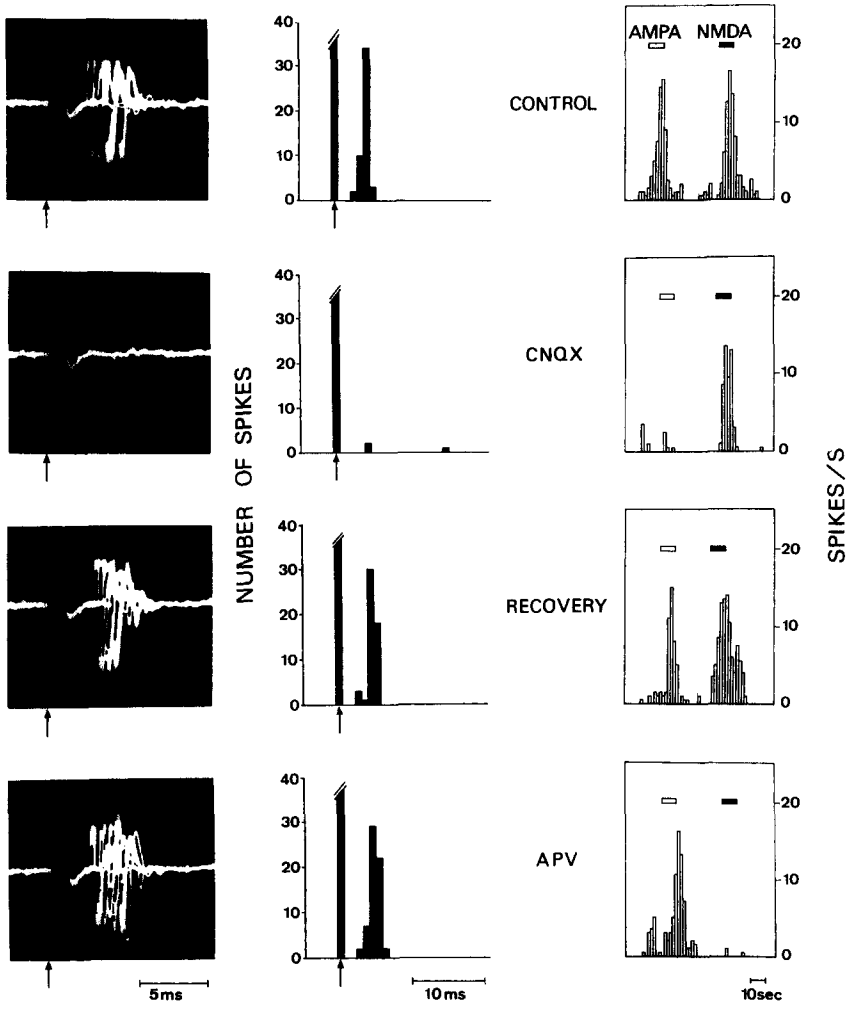

Fig. 5. Effects of CNQX and APV on a short latency excitatory response evoked by mediodorsal thalamic nucleus stimulation and on AMPA- and NMDA-induced activation of the same prefrontal cortex neuron in a ketamine-anaesthetized rat. The excitatory response to low frequency stimulation (0.3 Hz) of the mediodorsal thalamic nucleus is represented by 5 superimposed oscilloscope sweeps (left panel) and by peristimulus histograms (50 sweeps; middle panel). Rate histograms illustrate AMPA- and NMDA-evoked excitation of the same prefrontal cortex neuron (right panel). From top to bottom: responses under control conditions; the application of CNQX (50 nA) completely antagonized the mediodorsal thalamic nucleus- and AMPA-evoked excitations but did not affect the NMDA-evoked response; 3 min after the cessation of CNQX application, recovery of the excitatory response was observed; the application of APV (80 nA) did not affect the mediodorsal thalamic nucleus- and AMPA-evoked excitation but blocked the NMDA response. Horizontal bars indicate the duration of AMPA (10 nA) and NMDA (15 nA) iontophoretic applications. Arrows indicate the stimulus artefact.

activation of the mediodorsal thalamic nucleus-prefrontal cortex pathway were mainly blocked by CNQX and rarely by APV. Similarly, in ketamine-anaesthetized animals, these short latency excitatory responses were blocked in 91% of the cells by iontophoretic application of CNQX but not affected by APV (Table 2 and Fig. 5).

4. Discussion

Binding studies were the first to show that the rat prefrontal cortex was a cortical region particularly rich in AMPA and NMDA receptors, the two main types of ionotropic receptors for excitatory amino acids (Monaghan et al., 1984; Monaghan and Cotman, 1985; Sakurai et al., 1991). Molecular cloning and functional expression investigations have demonstrated that the pharmacologically defined AMPA and NMDA receptors are composed of heteromeric assemblies of subunit proteins (Hollmann and Heinemann, 1994). Recently, using antibodies which recognize different AMPA receptor subunits, the Glu_2 receptor subunit was found to be present in all cortical layers, and pyramidal neurons were shown to express Glu_1 receptor and $Glu_{2/3/4}$ receptor immunoreactivity (Martin et al., 1993). Immunostaining with antibodies against $NMDA_1$ receptor and $NMDA_{2A/B}$ receptor was observed on dendrites and synapses throughout the cerebral cortex. However, among postsynaptic densities, dense staining was more commonly seen with the $NMDA_{2A/B}$ receptor than with the $NMDA_1$ receptor antibody (Petralia et al., 1994). In the monkey, all neocortical cells possessing $Glu_{2/3}$ receptor subunits were also shown to be $NMDA_1$ receptor immunoreactive (Huntley et al., 1994). In the present study, in agreement with previous observations, all prefrontal cortex cells tested were activated following iontophoretic application of either AMPA or NMDA and these responses were blocked by their respective antagonists, CNQX and APV, suggesting that the two types of ionotropic glutamate receptors coexist on these cells.

EPSPs with both non-NMDA and NMDA components have been observed in prefrontal cortex slices following stimulation of the white matter or superficial layers (Sutor and Hablitz, 1989; Thomson et al., 1989; Hirsch and Crepel, 1990). As demonstrated in neocortical slices, using single-axon techniques, non-NMDA as well as NMDA receptors are involved in local circuits between pyramidal neurons (Jones and Baughman, 1988; Thomson and Deuchars, 1994). In agreement with these results, in the prefrontal cortex of halothane-anaesthetized rats, excitatory responses evoked by activation of recurrent collaterals from prefrontal cortex-mediodorsal thalamic nucleus neurons were blocked by CNQX and/or APV. However, a greater proportion of excitatory responses were blocked by APV than by CNQX, and these antagonists were both effective in a few cases only. A similar observation was made in the pre-cruciate cortex of halothane-anaesthetized cats where the recurrent EPSP in pyramidal neurons evoked by stimulating the pyramidal tract was more sensitive to APV (Herrling et al., 1990), suggesting a prominent contribution of NMDA receptors to these responses. At high concentrations, CNQX could interact with the glycine site of the NMDA receptors (Birch et al., 1989). Therefore, when the effect of CNQX on mediodorsal thalamic nucleus-evoked excitatory responses was investigated, it was always verified that the iontophoretic current used to block AMPA responses did not affect those of NMDA. In a few cases, cortical excitatory responses were only blocked by CNQX and not affected by APV, indicating that excitatory responses induced by activation of recurrent collaterals are not exclusively mediated by NMDA receptors. The contribution of AMPA receptors to the long latency responses evoked by mediodorsal thalamic nucleus stimulation was revealed in ketamine-anaesthetized rats since excitatory responses in these animals were, in most cases, blocked by CNQX and not by APV. As will be discussed more extensively later, this also indicates that ketamine (at a dose required for maintenance of anaesthesia) blocks the NMDA receptors contributing to synaptic transmission in the prefrontal cortex. Together, these data further confirm that recurrent collaterals from the prefrontal cortex-mediodorsal thalamic nucleus projecting neurons use an excitatory amino acid as the neurotransmitter and that both AMPA and NMDA receptors contribute to local circuit transmission in the prefrontal cortex.

As shown in vivo by extracellular and intracellular studies coupled with iontophoretic application of drugs, thalamocortical excitatory inputs predominantly involve AMPA receptors and few, if any, NMDA receptors (Tsumoto et al., 1986; Hagihara et al., 1988; Armstrong-James et al., 1993). Our data on the mediodorsal thalamic nucleus-prefrontal cortex pathway extend these observations since in both halothane (Pirot et al., 1994) and ketamine-anaesthetized rats (present study), the great majority of the short latency excitatory responses evoked by mediodorsal thalamic nucleus stimulation (0.3–1 Hz) were blocked by local application of CNQX and not by APV. Nevertheless, a contribution of NMDA receptors in mediodorsal thalamic nucleus-prefrontal cortex transmission cannot be completely excluded. Indeed, due to possible cell hyperpolarization induced by anaesthetic potentiation of inhibitory GABAergic transmission and to voltage-dependent Mg^{2+} block of the NMDA receptor channel (Franks and Lieb, 1994), NMDA receptor-mediated responses may not have been detectable with our experimental conditions.

General anaesthetics have a depressant action on excitatory synaptic transmission and some may interact with ligand-gated channels (Franks and Lieb, 1994). This has been demonstrated with the dissociative agent, ketamine, which largely exerts its anaesthetic effect by inhibiting NMDA receptors. Indeed, ketamine is a non-competitive NMDA antagonist which does not affect AMPA receptors (Anis et al., 1983; Thomson et al., 1985; Carlà and Moroni, 1992). In contrast, volatile agents such as halothane have no effect on NMDA receptor channels, and in addition AMPA receptors were also believed to be relatively insensitive to these agents (Franks and Lieb, 1994). However, according to recent observations, halothane may reduce AMPA receptor-mediated transmission. Indeed, halothane was shown to inhibit more potently currents activated by kainate than quisqualate or NMDA in cultured hippocampal neurons from mice (Peoples and Weight, 1992). Furthermore, investigations on the effects of general anaesthetics on AMPA or NMDA responses in mouse cortical wedges placed in a two-compartment bath have indicated that halothane preferentially antagonizes AMPA responses while ketamine selectively blocks NMDA responses (Carlà and Moroni, 1992). In agreement with these observations, our study demonstrated that, in doses required for maintenance of anaesthesia, ketamine and halothane affect differently excitatory synaptic transmission mediated through AMPA or NMDA receptors in the prefrontal cortex. Indeed, in halothane-anaesthetized rats, the long latency excitatory responses evoked by mediodorsal thalamic nucleus stimulation (3–10 Hz) were blocked by CNQX as well as by APV in most cases. In contrast, under ketamine anaesthesia these responses were only blocked by CNQX, confirming that this anaesthetic blocks the excitatory synaptic transmission mediated by NMDA receptors but preserves responses involving AMPA receptors. In addition, the proportion of cortical neurons which presented long latency excitatory responses to mediodorsal thalamic nucleus stimulation was smaller in halothane- than in ketamine-anaesthetized rats. This was also the case for the short latency excitatory responses induced by mediodorsal thalamic nucleus stimulation (0.3–1 Hz) which involve mainly, if not exclusively, AMPA receptors. Consequently, in the prefrontal cortex, halothane anaesthesia very likely depresses excitatory synaptic transmission mediated through AMPA receptors. Accordingly, the current-response curve indicated that prefrontal cortex cells are less sensitive to iontophoretic application of AMPA in halothane- than in ketamine-anaesthetized rats. Although it could be suggested from these data that halothane affects the AMPA receptor channel, the reduction of AMPA-mediated synaptic responses could also result from an action of this volatile anaesthetic on inhibitory GABAergic transmission. Indeed, general anaesthetics, including halothane, strongly potentiate the $GABA_A$ receptor-gated Cl^- currents, whereas ketamine has a weaker effect on these currents (Yeh et al., 1991; Lin et al., 1993). Finally, it also cannot be completely excluded that halothane and ketamine have distinct effects on voltage-gated Ca^{2+} channels involved in neurotransmitter release (Franks and Lieb, 1994).

Even though ketamine anaesthesia blocked cortical responses of the NMDA type resulting from mediodorsal thalamic nucleus stimulation, surprisingly, it reduced only slightly the sensitivity of prefrontal cortex cells to iontophoretic application of NMDA. Furthermore, local application of APV still decreased the activity of spontaneously active cortical cells in ketamine-anaesthetized animals, suggesting that part of the NMDA receptors were still active. As shown for cultured cortical neurons, NMDA receptors are present in high density ('hot spots') in synapses and in low density ('cold spots') in extrasynaptic regions, and glutamate may induce substantial currents through these extrasynaptic receptors when it is applied iontophoretically on a large region of the cell (Jones and Baughman, 1991). Accordingly, the activation of prefrontal cortex cells by iontophoretic application of NMDA could be due mainly to the activation of extrasynaptic receptors and, therefore, suggesting a difference in the sensitivity of the extrasynaptic and synaptic NMDA receptors to ketamine.

In conclusion, our results indicate that excitatory responses in the prefrontal cortex evoked by mediodorsal thalamic nucleus stimulation mediated through AMPA (mediodorsal thalamic nucleus-prefrontal cortex pathway) and/or NMDA (recurrent collaterals) receptors, are particularly sensitive to anaesthesia. Moreover, depending on the type of anaesthetic used, different responses may be obtained, emphasizing what care must be taken in the interpretation of data obtained in anaesthetized animals. Ketamine seems to be a more suitable agent for investigations of excitatory synaptic transmission involving AMPA receptors while halothane appears to be preferable for examining responses involving NMDA receptors.

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