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Noradrenaline induces IPSCs in rat medial septal/diagonal band neurons: involvement of septohippocampal GABAergic neurons

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1. The physiological and pharmacological actions of noradrenaline (NA) on neurons of the medial septum and diagonal band of Broca (MSDB) were examined using extracellular, intracellular and whole-cell patch-clamp recordings in an *in vitro* rat brain slice preparation.
2. In current- and voltage-clamp recordings with KCl- or potassium gluconate-containing electrodes, bath-applied NA increased the number of tetrodoxin- and bicuculline-sensitive synaptic events in > 80% of cholinergic- and GABA-type neurons tested. The NA-induced synaptic activity originated from GABAergic neurons located within the MSDB itself, as a similar effect occurred in brain slices in which the MSDB had been surgically isolated from neighbouring structures.
3. In antidromic studies, NA dose-dependently increased firing in a subpopulation of septo-hippocampal neurons with fast conducting fibres (mean conduction velocity, $1{\cdot}78 \pm 0{\cdot}10$ m s^{-1}; presumably GABAergic). The NA excitation was mimicked by the α_1-agonist phenylephrine (PE) and blocked by the α_1-antagonists prazosin and WB-4101, suggesting the presence of α_1-receptors on septohippocampal GABAergic neurons.
4. Similarly, in whole-cell recordings in both cholinergic- and non-cholinergic-type MSDB neurons, prazosin blocked the effects of NA and PE mimicked the effects of NA by inducing IPSCs with a similar amplitude distribution.
5. Consistent with the above findings, GABA-type neurons that responded directly to NA and PE with a prazosin-sensitive inward current were found within the MSDB.
6. In conclusion, NA, via α_1-adrenoceptors, excites MSDB septohippocampal GABAergic neurons and influences both septal and septohippocampal circuitry.

The medial septum/diagonal band of Broca (MSDB), which is part of the 'basal forebrain complex', provides major cholinergic (Lewis & Shute, 1967) and GABAergic (Köhler, Chan-Palay & Wu, 1984; Freund & Antal, 1988) inputs to the hippocampus, mainly via the fimbria/fornix (Lewis & Shute, 1967). The MSDB has been termed the 'pacemaker of the hippocampus' (see Stewart & Fox, 1990) as the septo-hippocampal projection is crucial for both the generation and the maintenance of hippocampal theta activity and related behaviours such as spatial learning (Bland, 1986). The MSDB itself receives numerous inputs. A major ascending noradrenergic projection originating from the rat locus coeruleus (LC) terminates in the MSDB (Moore, 1978; Vertes, 1988) and catecholaminergic terminals have been observed around both cholinergic and non-cholinergic neurons (Milner, 1991). More recently, direct contacts between noradrenaline terminals and septohippocampal neurons have been reported (Milner, Kurucz, Veznedaroglu & Pierce, 1995). Whether the NA fibres terminate exclusively on septohippocampal cholinergic neurons or also on septohippocampal GABAergic neurons is not known.

In behavioural studies, septal α-noradrenergic antagonism has been reported to produce a deficit in working memory performance in mice (Marighetto, Durkin, Toumane, Lebrun & Jaffard, 1989). The effect of septal NA on acetylcholine release in the hippocampus is unclear (Nilsson, Leanza & Bjorklund, 1992). Similarly, in electrophysiological studies conducted *in vivo*, both excitatory and inhibitory responses have been reported both following electrical stimulation of the LC (Segal, 1976) and after local injections of NA (Segal, 1974; Winokur & Beckman, 1978; Kim, Dudley & Moss, 1988). In the only *in vitro* brain slice study available, NA produced both inhibitory and excitatory effects in the rat MSDB (Zhadina & Vinogradova, 1984). Intracellular studies on the effects of NA on cholinergic and non-cholinergic neurons of the MSDB are lacking. Therefore, the goal of the present study was to examine the physiological and pharmacological actions of NA on electrophysiologically

characterized MSDB neurons and also on antidromically identified septohippocampal neurons. This goal was achieved using extracellular, intracellular and whole-cell recording techniques in an *in vitro*, rat brain slice preparation of the MSDB.

METHODS

Preparation of brain slices

Brain slices containing the MSDB were prepared from young adult male Sprague–Dawley albino rats (3–4 weeks old; body weight, 80–130 g) using methods detailed previously (Alreja & Aghajanian, 1995; Alreja, 1996). Briefly, rats were anaesthetized with chloral hydrate (400 mg kg^{-1} I.P.) and killed by decapitation. To enhance the yield of healthy cells, brain slices were prepared using artificial cerebrospinal fluid (ACSF) in which the NaCl was initially replaced with an equiosmolar concentration of sucrose (see Alreja & Aghajanian, 1995). The ACSF (pH 7·35–7·38), equilibrated with 95% O_2–5% CO_2, contained (mM): NaCl, 126; KCl, 3; NaH_2PO_4, 1·25; D-glucose, 10; $NaHCO_3$, 25; $CaCl_2$, 2; and $MgSO_4$, 2. Sucrose–ACSF contained equiosmolar sucrose instead of 126 mM NaCl. Following decapitation, the brain was removed and placed in a Petri dish containing sucrose–ACSF and trimmed to yield a small block containing the MSDB. Coronal or sagittal slices of ~500 μm thickness containing the MSDB were cut with a vibrating-knife microtome (Vibraslice, WP Instruments, Sarasota, FL, USA) and the slice transferred to the stage of a gas–liquid interface-type of brain slice chamber over which humidified 95% O_2–5% CO_2 flowed. The stage temperature was gradually raised from room temperature to 33 ± 0·5 °C over ~20 min. One to two hours later the slice was used for recording. The chamber was continuously perfused with normal ACSF at a rate of 1–2 ml min^{-1}. While most recordings were performed in coronal slices, sagittal slice preparations containing the dorsal fornix were used for the antidromic-activation studies.

In most recordings from coronal slices, the slice preparation also included the lateral septum, the bed nucleus of the stria terminalis, parts of the striatum and the ventral pallidum. However, in nine coronal slice preparations, the MSDB was isolated from all neighbouring structures (including the lateral septum) by knife cuts, such that the slice contained only the MSDB (stippled area in Fig. 1*A*). The purpose of these experiments was to study the effects of NA on MSDB neurons independent of any inputs from neighbouring structures (see Results).

Electrophysiological recordings were made primarily from the ventral portion of the medial septum and from the vertical limb of the diagonal band of Broca.

Intracellular recordings

Intracellular recordings were performed using sharp micro-electrodes (25–35 MΩ resistance) filled with 2 M KCl. All recordings were made using an Axoclamp-2A amplifier (Axon Instruments) either in the bridge mode or in discontinuous single-electrode voltage-clamp mode. In current-clamp recordings, the output signal was filtered at 10 kHz.

The cells selected for study had spike amplitudes of 70–100 mV. The electrophysiological criteria for identification of cholinergic and non-cholinergic type (presumably GABAergic) were based on those defined in the guinea-pig and rat septum by earlier workers (Griffith & Matthews, 1986; Segal 1986; Griffith, 1988; Markram & Segal, 1990). Spike durations were measured at half-spike amplitude. In spontaneously firing cells, these measurements were done at the resting potential, and in quiescent cells, firing was evoked by injecting a small amount of depolarizing current. Input resistance was calculated by measuring the instantaneous voltage following injection of a hyperpolarizing current. The effects of bath-applied NA on synaptic events were recorded only after chloride loading appeared complete (~10–20 mins).

Discontinuous single-electrode voltage-clamp recordings were performed using previously described methods (Alreja, 1996). The cells were voltage clamped at −60 mV. The input impedance of each cell was continually monitored by stepping the membrane potential to −65 or −70 mV for 1 s at 20 s intervals. The current and voltage signals were amplified and displayed on storage oscilloscopes and also continuously recorded on a chart recorder (Gould 2200).

Whole-cell recordings, acquisition and analysis of synaptic currents

Whole-cell patch-clamp recordings were performed using previously described methods (Alreja & Aghajanian, 1995). In brief, low-resistance (2·5–3·5 MΩ) patch pipettes were filled with a solution containing (mM): potassium gluconate, 120; Hepes, 10; K_4-BAPTA, 5; sucrose, 20; $CaCl_2$, 2·38; $MgCl_2$, 1; K_2ATP, 1; and GTP, 0·1 (pH 7·32–7·35). A few experiments were done with KCl- or CsCl-containing pipette solution. Since most recordings were performed with potassium gluconate-containing solutions (wherein the polarity of the IPSCs was normal and not reversed in the depolarizing direction), no attempts were made to block spontaneously occurring spikes, especially since spike characteristics were useful in the characterization of cholinergic and non-cholinergic neurons.

Synaptic currents were recorded using the continuous single-electrode voltage-clamp mode. The series resistance was continually monitored and cells were used for recording only if the series resistance was <6 MΩ. Series resistance compensation was not done. If the series resistance increased during the course of the experiment and caused significant reductions in the IPSC amplitudes, efforts were made to improve access by applying one of several manoeuvres (such as applying additional suction or slight positive pressure), failing which the experiment was discontinued.

Spontaneously occurring IPSCs (sIPSCs) were filtered at 3 kHz, amplified 100 times and digitized at 15 kHz (to minimize distortions in the fast rising phase of the synaptic currents) using the Digidata 1200 (Axon Instruments). With gluconate-containing electrodes, IPSCs were usually acquired at a holding potential of −60 mV (unless otherwise stated). The reversal potential of the IPSCs was estimated by observing the amplitude and polarity of the IPSCs in each cell at different holding potentials ranging from −60 to −120 mV. With Cl^--containing electrodes IPSCs were recorded at −90 mV. Ten 1 s sweeps of IPSCs were collected over 12 s for each experimental condition. Spontaneously occurring EPSCs were blocked by bath application of the NMDA and non-NMDA antagonists DL-2-aminophosphonovaleric acid (AP5; 50 μM) and 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX; 20 μM).

Off-line analysis of sIPSCs was performed using the commercially available computer software package Axograph 2·0 (Axon Instruments), wherein the traces were visually inspected for synaptic events and manually marked using the mouse-measure command. This method ensured that the analysis was not corrupted by any slight change in the noise level or by membrane fluctuations. If the background noise increased during the recording, the data from that cell were discarded. The data

generated from these measurements were used to plot cumulative probability amplitude and inter-event interval graphs, with each distribution normalized to a maximal value of 1. Cumulative probability plots obtained under different experimental conditions were compared using the non-parametric Kolmogorov–Smirnov (K–S) test, which estimates the probability that two cumulative distributions differ from each other by chance alone (Van Der Kloot, 1991; see Lupica, 1995). In comparing two sets of cumulative distributions, the K–S statistic, D, is the maximum distance between the two plots. The probability that the value of D arose by chance is given by:

$$P = Q_{KS} D[N_1 N_2/(N_1 + N_2)]^{1/2},$$

where N_1 and N_2 are the number of data points in the two distributions. The term $D[N_1N_2/(N_1 + N_2)]^{1/2}$ is designated λ. The K–S multiplier Q_{KS} which yields the above probability is calculated by the following equation:

$$Q_{KS}(\lambda) = 2\sum_{j=1}^{\infty}(-1)^{(j-1)}\exp(-2j^2\lambda^2).$$

The higher the value of P, the more likely that the compared curves are not different. The significance level for the K–S test was set at a conservative value of $P < 0{\cdot}01$. All numerical values are plotted as means ± standard error of the mean.

Antidromic activation of septohippocampal neurons and extracellular recordings

Extracellular recordings were made with glass micropipettes filled with 2 M NaCl (5–10 MΩ) and the fornix was stimulated using a bipolar Teflon-coated tungsten electrode. Septohippocampal projection neurons (SHNs) were identified by their antidromic response to electrical stimulation of the dorsal fornix (square pulses of 0·1–0·3 ms duration, 40–1000 μA) using the following criteria: fixed latency of activation, high frequency following and collision of the antidromic spikes with orthodromic spikes (Fig. 4). Similar criteria have previously been used to identify septohippocampal neurons *in vivo* (Lamour, Dutar & Jobert, 1984). In the case of quiescent cells, the collision test was performed after evoking orthodromic spikes with a brief application of NMDA, carbachol or 5-HT (agents that are known to excite MSDB neurons). The threshold of activation and the latency of antidromic activation were measured for each SHN and the latency measurement was used to compute the conduction velocity for each SHN. The distance between the stimulating and the recording electrodes was measured using a calibrated graticule located in the eyepiece of the dissection microscope.

Pharmacological studies were conducted using the extracellular recording technique. Concentration–response curves for NA (10–100 μM) were plotted before and after treatment with a single dose of antagonist (10 nM prazosin or 40 nM WB-4101, bath applied for 20 min). Three concentrations of the agonist were tested for each cell, with the highest concentration tested last. Each cell was normalized to its own maximum excitation, and the EC_{50} values calculated using the non-linear least-squares fitting algorithm as implemented in SigmaPlot (Jandel Scientific, Corte Madera, CA, USA). To allow comparison of the potency of the antagonists in the present study with published pK_B values reported in binding studies, the pA_2 values for the antagonists were calculated for each cell (since pK_B always corresponds to pA_2). The pA_2 value, which is equal to the negative logarithm of the molar concentration of the antagonist that produces a 2-fold shift to the right in the agonist dose–response curve, was calculated using the following equation:

$$\log(\mathrm{dr} - 1) = \log[B] - \log K_B,$$

where, B is the concentration of the antagonist and $pA_2 = pK_B$ when the dose ratio (dr) is equal to 2. The dose ratio was the EC_{50} for the agonist in the presence of antagonist divided by the EC_{50} obtained in the absence of antagonist.

Reagents

All constituents of the ACSF and patch pipette solution were obtained from Mallinckrodt and Sigma, respectively. The tetrapotassium salt of BAPTA was obtained from Molecular Probes. Bicuculline methiodide and tetrodotoxin (TTX) were obtained from Sigma. Noradrenaline bitartrate, phenylephrine, prazosin, WB-4101, CNQX and AP5 were obtained from Research Biochemicals Inc.

All drugs were diluted in ACSF from previously prepared stock solutions that were prepared in water (unless mentioned otherwise) and stored at −20 °C. Sodium metabisulphite (10 mM) (Sigma) was added to 20 mM noradrenaline stock solution to prevent oxidation. All drugs were bath applied by turning a three-way valve which switched from ACSF to the test solution. The turnover time of the recording chamber was less than 30 s.

RESULTS

NA induces inhibitory synaptic activity in both cholinergic and non-cholinergic MSDB neurons

In intracellular and whole-cell current-clamp recordings, performed with KCl-containing electrodes, bath-applied NA increased the number of depolarizing synaptic potentials in electrophysiologically distinct MSDB neurons (Fig. 1). This increase was seen in 82·1% of the cells tested (23/28 cells). In whole-cell recordings with a potassium gluconate-containing electrode, this effect of bath-applied NA was seen as an increase in the number of hyperpolarizing synaptic potentials and occurred in 83·7% of the cells tested (31/37 cells). These observations suggested that the depolarizing synaptic potentials recorded with Cl^--containing electrodes may in fact be reverse IPSPs (see below).

As mentioned above, the NA-induced increase in synaptic activity was observed in electrophysiologically distinct MSDB neurons. In intracellular recordings with KCl-containing electrodes, NA increased the number of reverse IPSPs in 20/24 neurons tested. Five of these neurons were electrophysiologically identified as cholinergic type and the remaining fifteen as non-cholinergic (presumably GABAergic). This characterization was based on criteria previously defined by other investigators (Griffith & Matthews, 1986; Griffith, 1988; Markram & Segal, 1990; see Methods). Cells classified as cholinergic type (e.g. Fig. 1*B*) had broad spikes (spike duration, 1·2 ± 0·2 ms; $n = 5$) and prominent slow after-hyperpolarizations (AHPs) but lacked a prominent depolarizing sag in the electronic response to a hyperpolarizing pulse. These cells had a mean input resistance of 115 ± 25 MΩ. The remaining fifteen cells,

classified as non-cholinergic type (e.g. Figs 1*C*, 2 and 6), had shorter duration spikes ($0{\cdot}4 \pm 0{\cdot}02$ ms) and prominent fast AHPs and displayed depolarizing sags on hyperpolarization. These cells on average had a lower input resistance ($65{\cdot}5 \pm 8{\cdot}1$ MΩ). Out of the four neurons which did not respond to NA, three were classified as non-cholinergic and one as cholinergic.

Similarly, in whole-cell recordings with potassium gluconate-containing electrodes, NA increased the number of IPSCs in 83·7% of the neurons tested (31/37 cells). Out of the thirty-seven neurons tested, fifteen were classified as cholinergic and the remaining twenty-two as non-cholinergic type (presumably GABAergic) as per the criteria mentioned above. NA increased IPSCs in 9/15 cholinergic neurons and

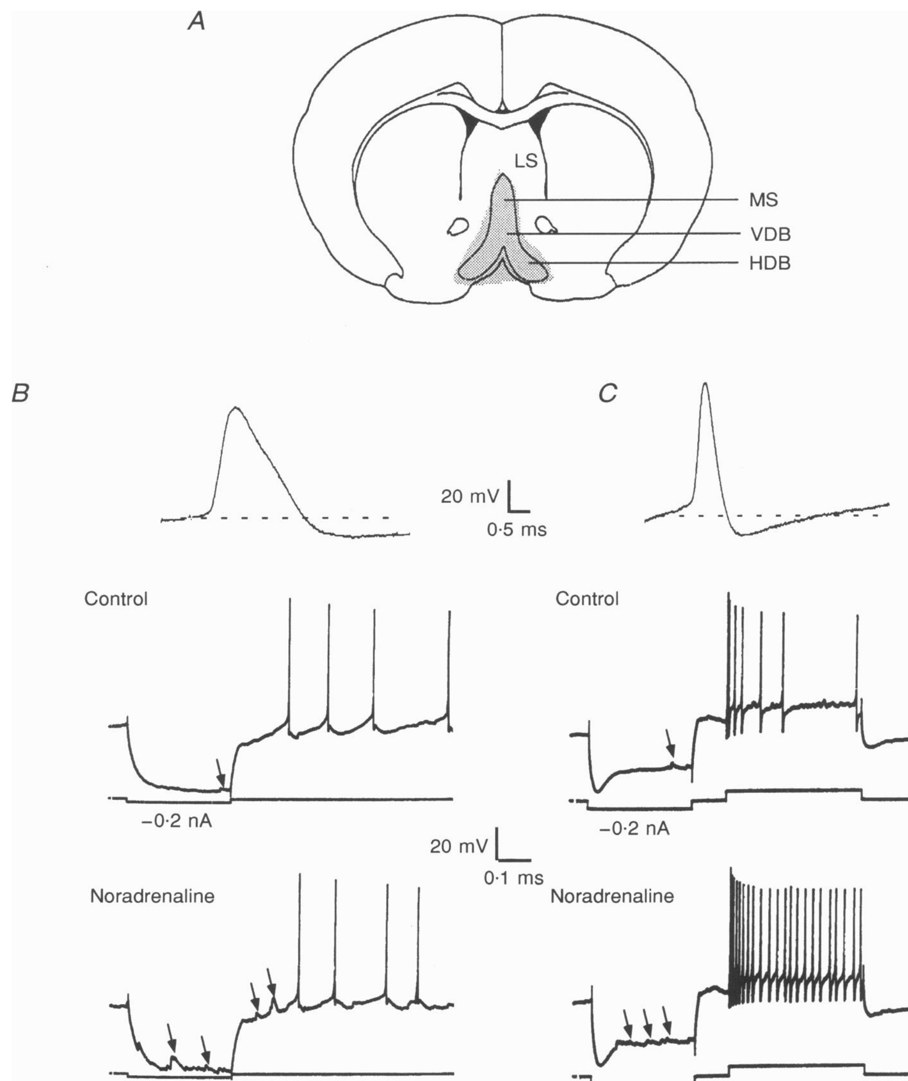

Figure 1. Bath-applied noradrenaline increases synaptic activity in both cholinergic- and non-cholinergic-type MSDB neurons

A, the slice preparation was restricted to the MSDB (stippled area) and did not contain any of the neighbouring structures such as the lateral septum (LS) (MS, medial septum; VDB, vertical limb of the diagonal band; HDB, horizontal limb of the diagonal band). *B* and *C*, intracellular recordings with KCl-containing electrodes from two electrophysiologically distinct MSDB neurons. *B*, bath-applied NA (100 μM) increased depolarizing synaptic potentials (arrows) in a spontaneously firing broad-spiked cholinergic-type MSDB neuron (spike duration, 1·2 ms). Note the prominent slow after-hyperpolarization, which is characteristic of septal cholinergic neurons. *C*, NA also increased the number of depolarizing synaptic potentials in a sharp-spiked non-cholinergic-type (presumably GABAergic) MSDB neuron (spike duration, 0·38 ms, resting membrane potential −68 mV). Note the prominent fast after-hyperpolarization and the pronounced depolarizing sag (in response to the hyperpolarizing pulse) that are characteristic of MSDB GABAergic neurons. Note that NA increased the basal firing rate in the GABA-type but not in the cholinergic-type neuron. Additionally, since the slice preparations contained only the MSDB, the NA-induced synaptic activity originated from within the MSDB.

21/22 non-cholinergic neurons. Thus, NA-excited neurons of the MSDB appear to make local synaptic contacts with both cholinergic and non-cholinergic MSDB neurons.

NA-induced synaptic activity originates from neurons within the MSDB

Theoretically, the NA-induced synaptic activity could originate from neurons within the MSDB or from other brain structures present in the slice preparation. In order to determine whether the NA induced increase in synaptic activity originated from neurons within the MSDB, we tested the effects of NA in brain slices where the MSDB had been surgically isolated from all neighbouring structures such as the lateral septum (stippled area in Fig. 1*A*). NA-induced a similar increase in synaptic activity in both types of slice preparations. Thus, 82·9% of the neurons responded

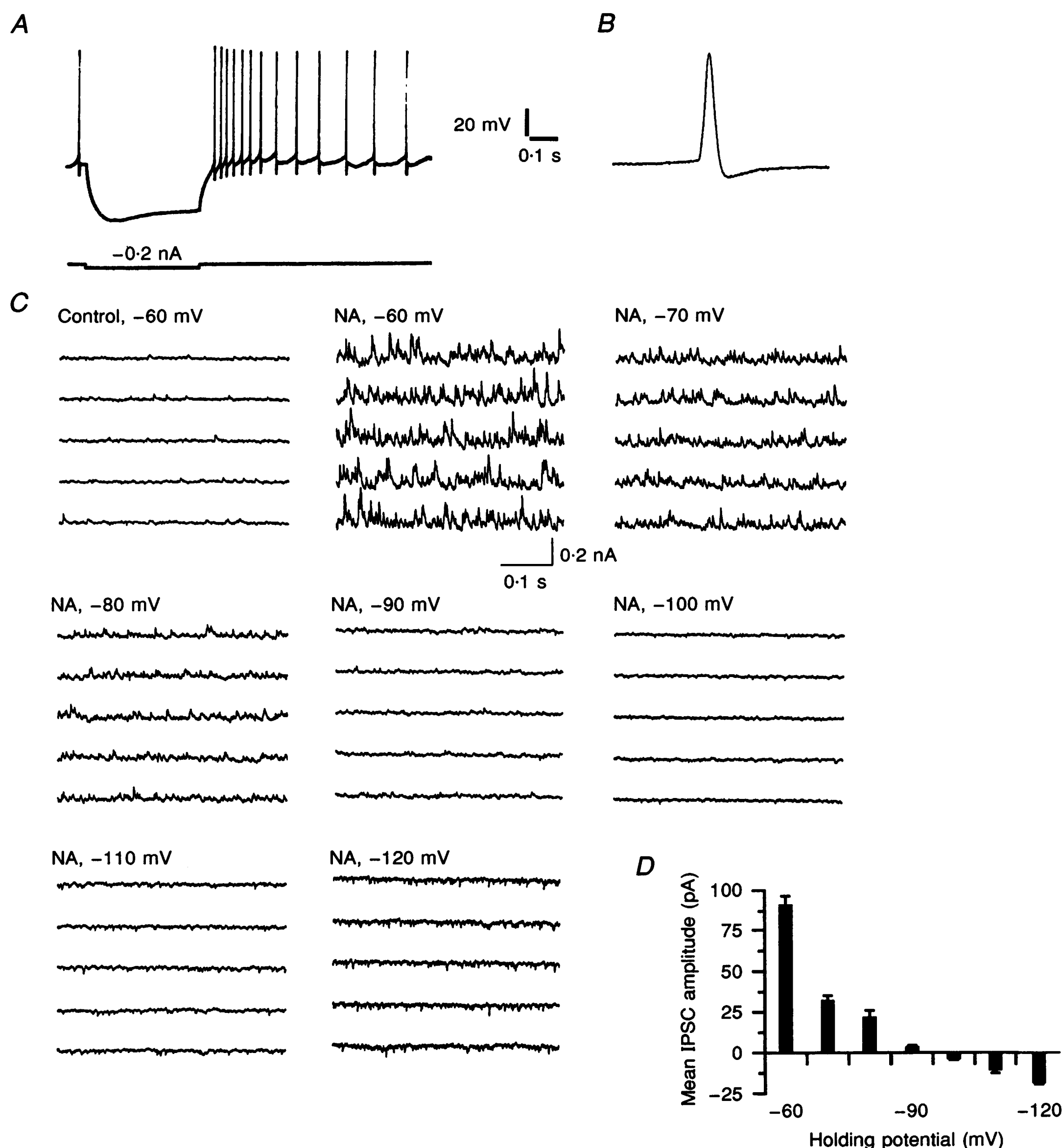

Figure 2. Noradrenaline-induced IPSCs reverse polarity near the chloride equilibrium potential

A and *B*, whole-cell current-clamp recording from a GABA-type MSDB neuron with a potassium gluconate-containing patch electrode. Note the fast after-hyperpolarization and the short-duration spike. *C*, consecutive 0·5 s traces show spontaneously occurring IPSCs recorded under different experimental conditions. The first sets of traces show IPSCs recorded at −60 mV under control conditions. Subsequent sets of traces show IPSCs recorded in presence of NA at different holding potentials (ranging from −60 to −120 mV). Note the changes in amplitude and polarity of the synaptic currents at different holding potentials. *D*, bar chart summarizing the data shown in *C*. The mean IPSC amplitude recorded in the presence of NA is plotted against different holding potentials. Note that the mean amplitude of the NA-induced IPSCs decreased at more negative holding potentials and reversed polarity between −90 and −100 mV. This experiment was done in presence of 20 μM CNQX and 50 μM AP5 to block excitatory synaptic currents.

to NA with an increase in synaptic activity in slices that contained the MSDB and neighbouring structures (39/47 cells tested in 29 slices). Similarly, 85·7% of the cells showed a response to NA in slices that contained only the MSDB (12/14 cells tested in 9 slices). This suggested that the NA-induced synaptic activity originates from neurons present within the MSDB itself. Of the brain regions neighbouring the MSDB, the lateral septum was of special interest as, until recently, it was believed to provide a massive GABAergic input to the MSDB (Leranth, Deller & Buzsaki, 1992).

NA-induced synaptic activity is blocked by bicuculline or tetrodotoxin

To analyse further the NA-induced changes in synaptic activity, whole-cell voltage-clamp recordings were performed in electrophysiologically distinct MSDB neurons using low-resistance patch electrodes that contained either potassium gluconate ($n = 37$) or KCl ($n = 3$) or CsCl ($n = 3$) as the major anion. As mentioned above, with gluconate-containing electrodes an increase in inhibitory synaptic currents was observed following bath application of NA (Figs 2 and 3*A*), whereas with chloride-containing electrodes the NA-induced synaptic currents were reversed in polarity (Fig. 3*C*). The amplitude and polarity of the NA-induced synaptic currents

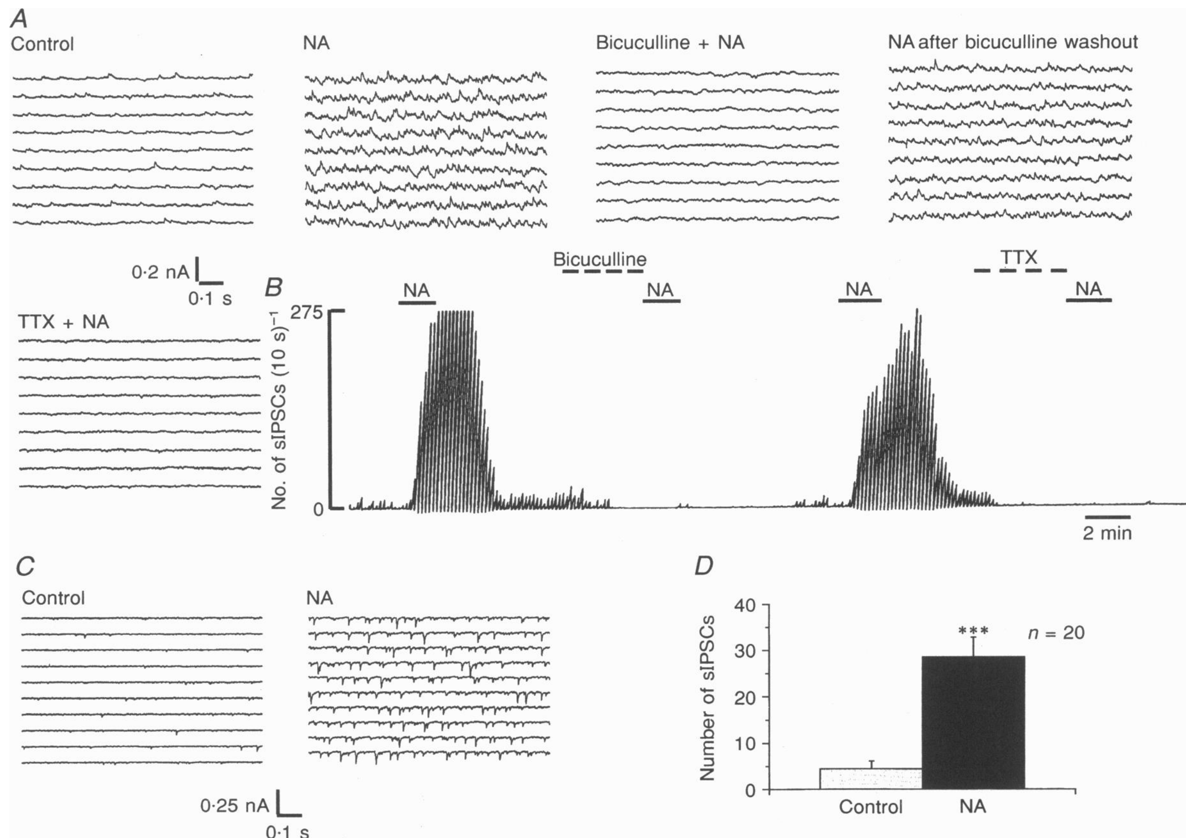

Figure 3. Bicuculline and tetrodotoxin (TTX) block noradrenaline-induced synaptic currents

A, whole-cell voltage-clamp recording from a MSDB neuron with a potassium gluconate-containing patch electrode. Consecutive 0·5 s traces show spontaneously occurring IPSCs recorded at −60 mV under different experimental conditions. The first set of traces shows IPSCs recorded under control conditions. Subsequent sets of traces show the effect of bicuculline and TTX on NA-induced IPSCs. Observe that bicuculline (10 μM) reversibly blocked NA-induced IPSCs, which were subsequently blocked by bath application of TTX (2 μM). *B*, chart record from the same cell showing the frequency of sIPSCs during the course of the experiment. Note that bicuculline and TTX blocked the effect of NA. *C*, whole-cell voltage clamp recording from a MSDB neuron with a CsCl-containing patch electrode. EPSCs were blocked using glutamate antagonists (see Methods). Consecutive 0·5 s traces show spontaneously occurring IPSCs recorded at −90 mV before and after bath application of NA. Note the reversed polarity of the IPSCs. *D*, bar chart summarizing the effect of NA on the frequency of sIPSCs recorded in 20 cells. NA produced a 6·5-fold increase in the frequency of sIPSCs ($P < 0·001$; Student's t test).

also varied with changes in E_{Cl}. Thus, in recordings with gluconate-containing electrodes (calculated $E_{Cl} = -80$ mV), the amplitude of the NA-induced synaptic currents decreased at more negative holding potentials and reversed polarity between −80 and −100 mV in all the cells tested. Figure 2 is an example of one such neuron in which the NA-induced IPSCs reversed between −90 and −100 mV. Presumably, the failure of all IPSCs to reverse precisely at the calculated Cl^- reversal potential arises from inadequate voltage clamping of distant dendritic synapses (Spruston, Jaffe & Johnston, 1994). In recordings with chloride-containing electrodes (calculated $E_{Cl} = 1{\cdot}5$ mV), the amplitude of the NA-induced reverse IPSCs increased at more negative holding potentials (not shown); the precise reversal potential of the synaptic currents with Cl^--containing elctrodes was not measured.

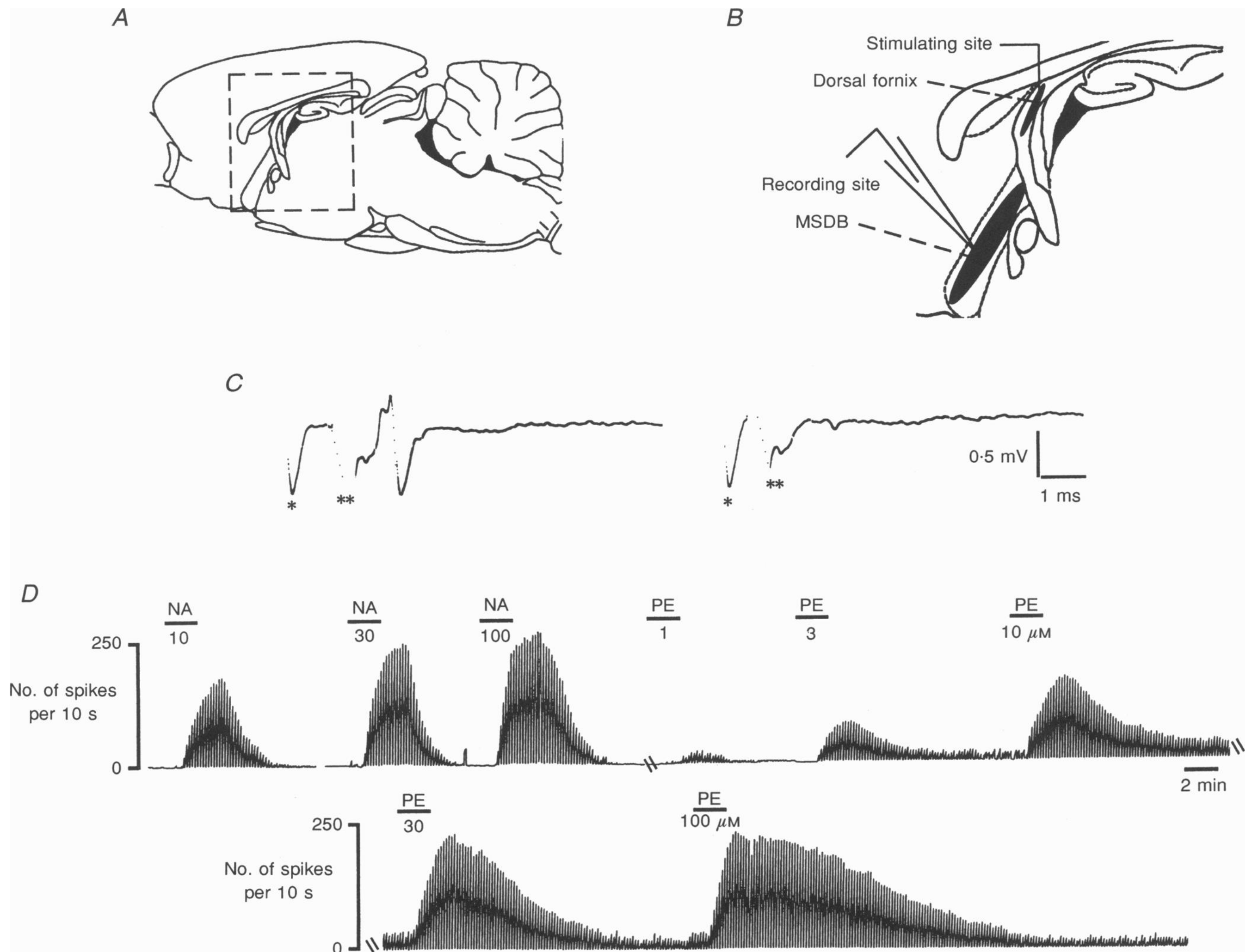

Figure 4. Noradrenaline and phenylephrine excite antidromically activated septohippocampal neurons

A and *B*, sagittal section through the rat brain, showing the septal area. The boxed area is enlarged in *B* and shows the medial septum/diagonal band (MSDB), which was the recording site. For antidromic activation of septohippocampal neurons, the stimulating electrode was placed in the dorsal fornix as it conveys both cholinergic and GABAergic MSDB fibres to the hippocampus. *C*, extracellular recording from an antidromically activated septohippocampal neuron. Since, this neuron was quiescent at rest, NA (see *D* below) was used to evoke firing (*) and the oscilloscope was triggered with the NA-evoked spike, the dorsal fornix was stimulated (**) 2 ms later and an antidromically activated spike was obtained after a latency of 1·25 ms (left trace). This cell was classified as GABA type based on the calculated conduction velocty of 1·6 m s^{-1}. Right trace shows a positive collision test, wherein the cell could not be activated antidromically when the dorsal fornix was stimulated 1 ms after the triggering spike (approximately half the antidromic latency). Stimulation current, 160 μA, 300 μs. *D*, a chart record trace from the antidromically activated neuron shown in *C*, above. Note that NA produced a reversible and dose-dependent increase in firing. This effect was dose-dependently mimicked by the α_1-agonist phenylephrine (PE) and a maximal effect was observed at a concentration of 30 μM.

Since the MSDB contains a large population of GABAergic neurons, we tested the effect of bicuculline, a $GABA_A$ antagonist, on the Cl^--mediated synaptic activity. In recordings with KCl or potassium gluconate-containing electrodes, both bicuculline (Fig. 3*A* and *B*; $n = 7$) and tetrodotoxin, a fast sodium channel blocker (Fig. 3*A* and *B*; $n = 5$), blocked NA-induced changes in synaptic activity. This suggested that the NA-induced changes were both trans-synaptic and GABAergic in nature. Thus, the NA-induced spontaneous synaptic events are not true miniature inhibitory postsynaptic currents (mIPSCs) reflecting quantal release, but in fact correspond to synaptic release caused by firing of GABAergic neurones. We have adopted the term 'spontaneous' to describe these events (sIPSCs); thus the term sIPSC simply means the synaptic currents have not been evoked via exogenous electrical stimulation. It should also be mentioned that in the present study, we did not observe any effects of NA on TTX-insensitive miniature postsynaptic currents (mPSCSs).

Since sIPSCs seen in the presence of NA are often larger in amplitude (see Fig. 3*A*), and could result from a change in input resistance of the postsynaptic cell, we performed three experiments with CsCl-filled electrodes and observed a similar effect (Fig. 3*C*). Thus the effect of NA on synaptic activity does not result from an improved space clamping of ongoing synaptic activity. The results of the above-described experiments therefore indicate that NA excites GABAergic neurons located within the MSDB which, in turn, make local synaptic contacts both with cholinergic and with other GABAergic neurons within the MSDB.

Effect of NA on antidromically activated septo-hippocampal neurons (SHNs)

Since a subpopulation of MSDB GABAergic neurons projects to the hippocampus (Freund & Antal, 1988; Freund, 1989), we tested the effect of NA on antidromically activated septo-hippocampal neurons. These experiments were performed in a sagittal brain slice preparation that contained the dorsal

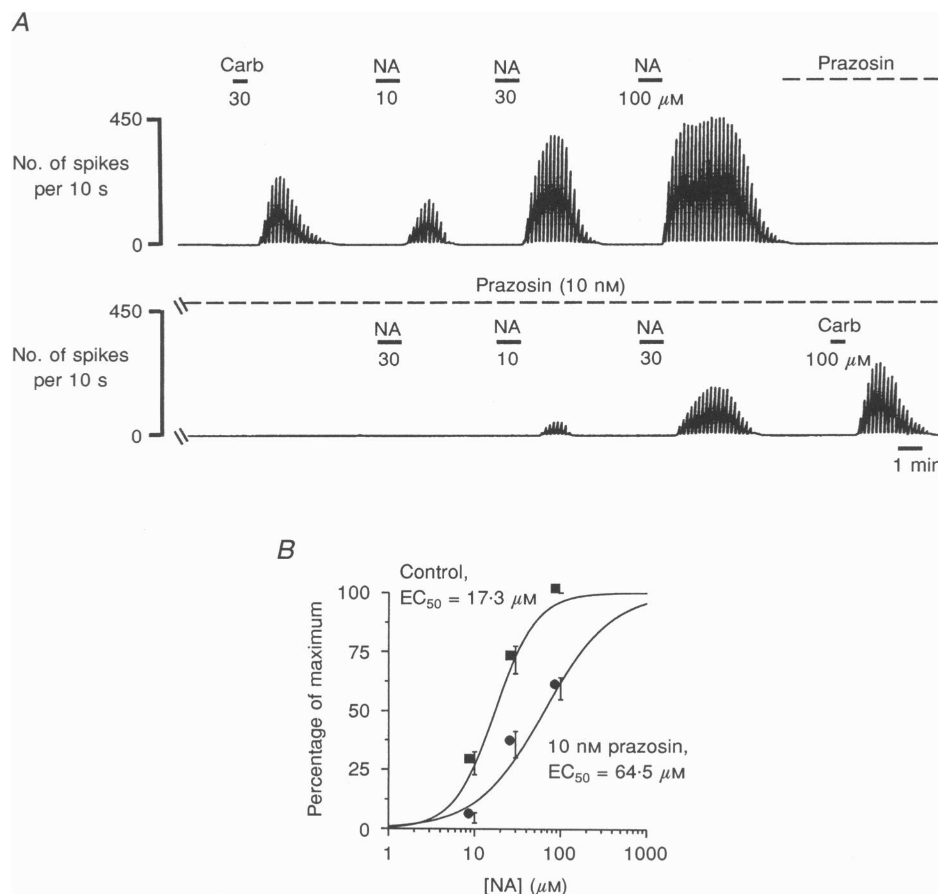

Figure 5. Prazosin, an α_1-antagonist, blocks noradrenaline-induced excitation

A, extracellular recording from an MSDB neuron showing that bath-applied NA produced a dose-dependent increase in cell firing. Following a 20 min bath application of prazosin, the response to NA was markedly reduced. A pA_2 value of 9·22 was obtained for this cell. Note that the excitatory response to carbachol (Carb), a cholinergic agonist, was not affected by prazosin. *B*, concentration–response curve for NA before and after application of 10 nM prazosin averaged from 11 cells. Each cell was normalized to its own maximal reponse (i.e. the response to 100 μM NA under control conditions). Note that prazosin produced a rightward shift in the NA dose–response curve. Error bars represent S.E.M.

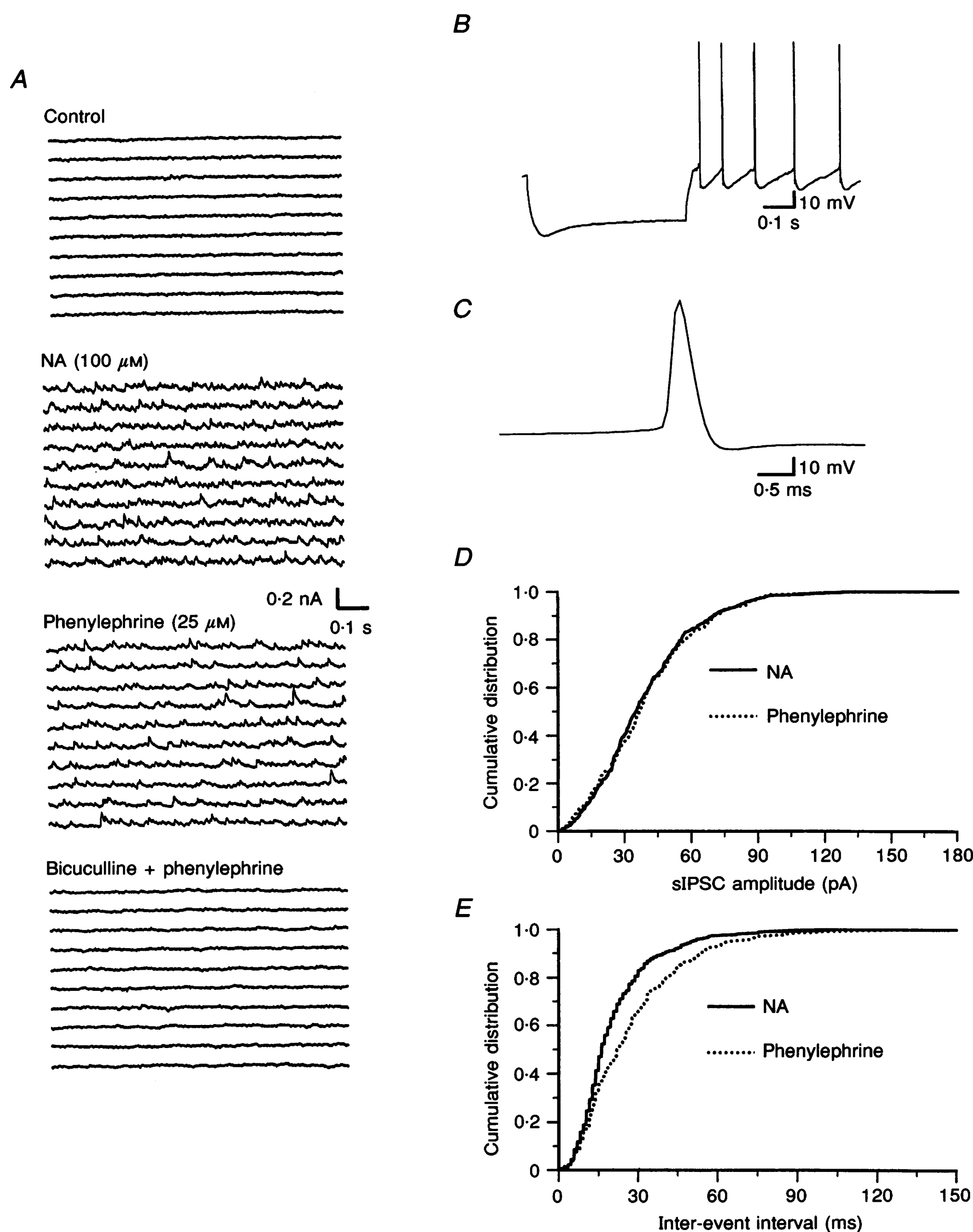

Figure 6. Noradrenaline- and phenylephrine-induced IPSCs have similar amplitude distributions

A, consecutive 1 s traces show spontaneously occurring IPSCs recorded at a holding potential of −60 mV under different experimental conditions. These whole-cell recordings were made in a GABA-type MSDB neuron with a potassium gluconate-containing patch electrode. Near-maximal concentrations of NA increased synaptic activity. A near-maximal concentration of PE (an α_1-agonist, phenylephrine) also increased synaptic activity. Bicuculline, a $GABA_A$ antagonist, blocked PE-induced increase in synaptic activity (bottom trace). Bicuculline also blocked NA-induced increase in IPSCs (not shown). *B* and *C* show current-clamp recordings from the same neuron. Note the depolarizing sag in response to a hyperpolarizing pulse (−0·2 nA; *B*) and the short duration 0·35 ms spike (*C*), that are characteristic of GABAergic MSDB neurons. *D* and *E*, cumulative amplitude and frequency distributions of sIPSCs constructed from data shown in *A*. NA- and PE-induced IPSCs had the same amplitude distribution ($P > 0{\cdot}5$, Kolmogorov–Smirnov (K–S) test; but a different frequency distribution ($P < 0{\cdot}0001$, 480 events analysed for NA and 349 events for PE). Note that with PE the frequency distribution was shifted to the right with larger inter-event intervals. This is consistent with the reported partial agonistic nature of PE.

fornix (Fig. 4A and B), as both septohippocampal cholinergic and GABAergic fibres travel to the hippocampus via the dorsal fornix. The stimulating electrode was placed in the dorsal fornix, and antidromically activated units were searched for in the MSDB using an extracellular electrode. In these recordings, a subpopulation of antidromically activated SHNs with fast conducting fibres (mean conduction velocity, 1.78 ± 0.10 m s^{-1}; $n = 58$), were found to be reversibly and dose-dependently excited by bath-applied NA. Figure 4C is an example of one such neuron. Neurons that were not excited by NA had a mean conduction velocity of 1.2 ± 0.18 m s^{-1} ($n = 6$). The short activation latency and the resultant high conduction velocity of the NA-responsive neurons suggested that these neurons may be GABAergic projection neurons (see Discussion).

Pharmacology of the NA-mediated excitation

In order to determine the receptor subtype(s) mediating the NA-induced excitatory responses in the MSDB, pharmacological studies were undertaken. In various brain regions, both the α_1- and the β-adrenoceptors have been shown to mediate excitatory responses to NA. Since receptor-binding

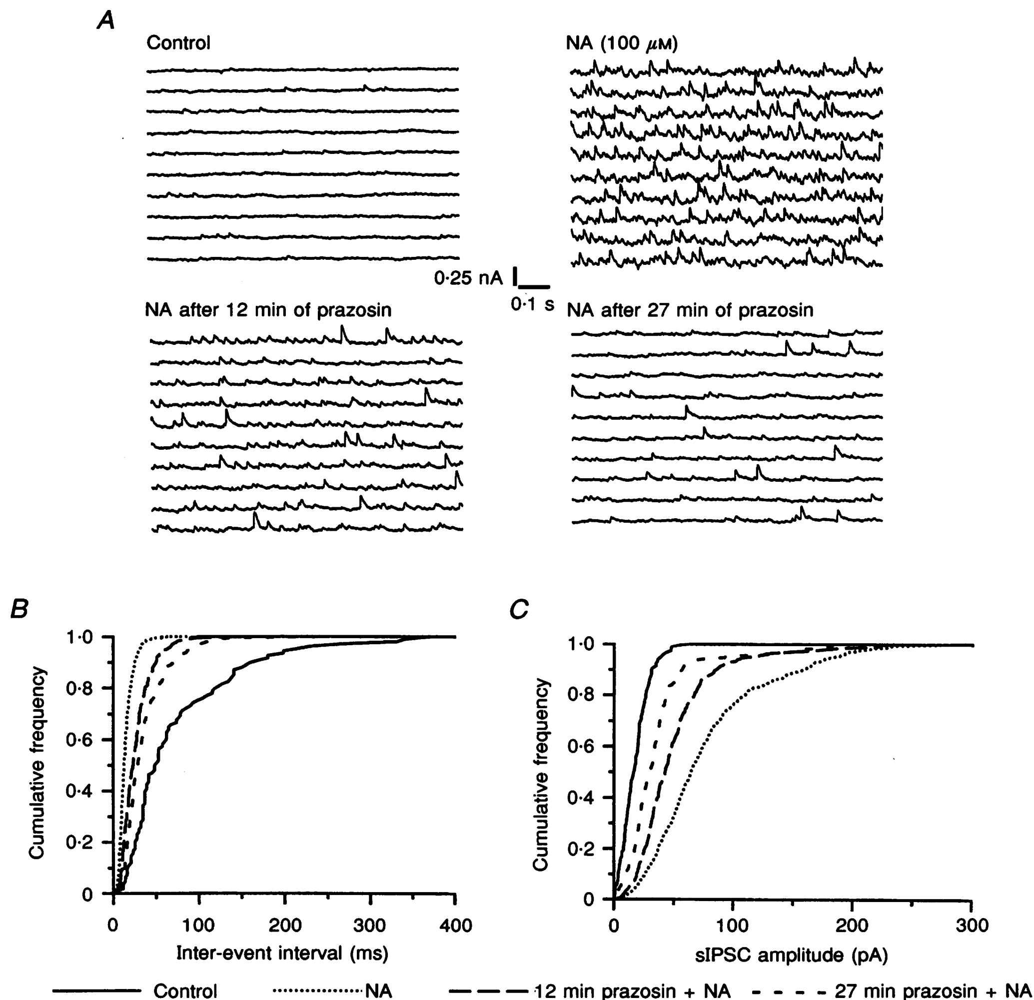

Figure 7. The α_1-antagonist, prazosin, blocks noradrenaline-induced increase in IPSCs

A, consecutive 1 s traces show spontaneously occurring IPSCs recorded at a holding potential of −60 mV with a patch electrode containing potassium gluconate. Note that the NA-induced increase in IPSCs was progressively blocked by prior treatment with the α_1-antagonist prazosin. B, cumulative inter-event interval distribution for sIPSCs shown in A. Note that prazosin produced a progressive shift in the curves moving towards longer inter-event intervals and closer to control values. Both the 12 min and 27 min curves were significantly different from the NA curve taken prior to application of prazosin ($P < 0.001$; K–S statistic). Also note that after 27 min of prazosin, the response to 100 μM NA (which produces a near maximal response) was further blocked. C, cumulative amplitude distribution for sIPSCs shown in A. Note the progressive shift in the curves after application of prazosin moving towards lower amplitudes and closer to control values. Both the 12 min and 27 min curves were significantly different from the NA curve taken prior to application of prazosin ($P < 0.001$; K–S statistic; 684 events analysed for NA alone, 368 events after 12 mins of prazosin and 268 events after 27 min). In this cell the NA-induced IPSCs reversed polarity between −90 and −100 mV (not shown).

(Palacios, Hoyer & Cortes, 1987) and mRNA studies have demonstrated the presence of α_1-receptors in the septal area (Pieribone, Nicholas, Dagerlind & Hokfelt, 1994) we tested the effect of α_1-selective agents on MSDB neurons. Some of these experiments were performed on antidromically activated SHNs. In extracellular recordings, NA produced a reversible and dose-dependent increase in cell firing with a mean EC_{50} of 17·3 μM (Fig. 5*A* and *B*; $n = 11$). A 20 min bath application of the α_1-antagonist prazosin (10 nM), while not having any effect of its own (Fig. 5*A*), produced a 3·75-fold shift to the right in the NA dose–response curve (Fig. 5*B*, $EC_{50} = 64·5$ μM; $n = 11$; 6/11 neurons were antidromically confirmed to be SHNs; mean conduction velocity, $2·22 \pm 0·47$ m s^{-1}). The pA_2 values for prazosin calculated individually for each cell ranged from 8·06 to 9·22 with a mean pA_2 of $8·53 \pm 0·12$. Similarly, WB-4101 (40 nM) which is also an α_1-antagonist, blocked the excitatory effects of NA in 3/4 cells tested (mean $pA_2 = 8·18 \pm 0·34$; $n = 3$).

The excitatory effects of NA were mimicked by the α_1-agonist phenylephrine (PE) in all the eleven neurons tested (Fig. 4*D*); 6/11 neurons were antidromically confirmed to be SHNs (mean conduction velocity, $2.08 \pm 0·23$ m s^{-1}). The dose–response relationship to PE was studied in three neurons and an EC_{50} of $4·0 \pm 0·85$ μM was obtained. In one cell, PE produced an excitation with an unusually high EC_{50} of 21·7 μM. Similar to NA, the excitatory effect of PE was also blocked by prior treatment with prazosin ($n = 8$) or WB-4101 ($n = 1$). Thus, these studies indicated a role for α_1-adrenoceptors in mediating the excitatory effects of NA in septohippocampal neurons with fast conducting fibres (> 2 m s^{-1}; presumably, GABAergic).

In whole-cell patch-clamp recordings, the excitatory effects of NA on GABAergic IPSCs were also found to be mediated via α_1-receptors. The effect on synaptic activity of a near-maximal concentration of PE (25 μM) was compared with

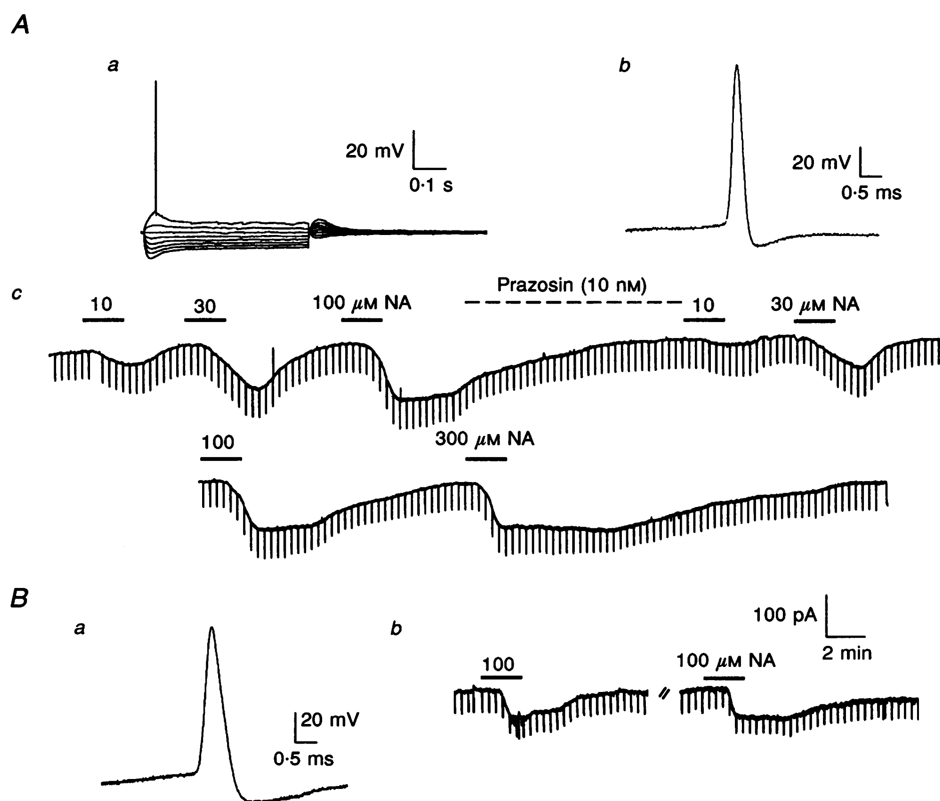

Figure 8. Noradrenaline induces a direct TTX-insensitive, α_1-receptor-mediated inward current in GABA-type MSDB neurons

A a and *A b*, whole-cell current-clamp recording from a GABA-type MSDB neuron taken prior to bath application of TTX (spike duration, 0·23 ms). Also note the pronounced depolarizing sag in response to the hyperpolarizing pulses (0·1 nA steps). *A c*, voltage-clamp recording from the same cell showing the dose response to NA in the presence of TTX. The cell was voltage clamped at −60 mV (holding current, +0·12 nA). NA produced an inward current with an EC_{50} of 11·3 μM; 100 μM NA produced a 340 pA inward current. Prazosin (10 nM, 10 min), an α_1-antagonist, blocked NA-induced inward current with a pA_2 of 8·27. *Ba* and *Bb*, effect of repeated applications of NA in another GABA-type neuron; spike duration, 0·46 ms. NA (100 μM) produced a 100 pA inward current at a holding potential of −60 mV (holding current, 0·01 nA). A second application of NA, tested 26 min later, also produced a similar response. Input conductance was measured in both cells by stepping the membrane potential to −65 mV for 1 s every 20 s.

that of a near-maximal concentration of NA (100 μM) in three GABA-type neurons. Phenylephrine mimicked the effect of NA and increased IPSCs in all three GABA-type neurons. Figure 6 is an example of one such experiment performed in a GABA-type neuron. Both NA and PE increased the number of bicuculline-sensitive IPSCs. Cumulative distribution curves showed that NA- and PE-induced IPSCs had a statistically similar amplitude distribution ($P > 0{\cdot}5$, K–S test), suggesting that the two agonists act on similar populations of GABAergic neurons. However, the frequency distribution for the synaptic currents recorded was significantly different ($P < 0{\cdot}001$) with fewer events being recorded with PE; this presumably occurs as a result of the known partial agonistic properties of PE.

The effect of prazosin on NA-induced synaptic activity was tested in three neurons (1 cholinergic and 2 GABA-type). In all three neurons, the NA-induced increase in IPSCs were progressively blocked by prazosin (Fig. 7). Statistically significant differences ($P < 0{\cdot}001$) were observed in both the frequency and the amplitude distribution of NA-induced IPSCs after treatment with prazosin, wherein prazosin produced a time-dependent shift in these curves.

Thus, these experiments indicated that NA-induced increases in GABAergic IPSCs are mediated via α_1-subtype of adrenoceptors.

NA-induced excitation of MSDB GABA-type neurons

Based on the above observations, one would predict the presence of GABAergic neurons in the MSDB that would be directly excited by NA. Accordingly, in a subpopulation of MSDB neurons, NA produced a direct, TTX-insensitive inward current that was associated with a decrease in apparent input conductance (mean amplitude, 136 ± 36 pA, $n = 9$). These neurons had short-duration spikes (0·4 ± 0·02 ms), a depolarizing sag and a low input resistance (< 100 MΩ). PE mimicked the NA-induced inward current in all three neurons tested. In the four neurons tested, the α_1-antagonist prazosin also blocked the NA-induced inward current. In two neurons, pA_2 values for prazosin were calculated using three concentrations of NA. Prazosin (10 nM) blocked NA-induced inward current with a mean pA_2 value of 8·7 (Fig. 8C). These values are comparable with those obtained in the extracellular studies mentioned above.

A small inward current (range, 10–60 pA; mean current, 32 ± 5·90 pA; $n = 8$) with either no clear-cut change in conductance or an increase in input conductance was observed in 8/13 cholinergic-type neurons tested. These neurons had a mean spike duration of 1·01 ± 0·1 ms. In the two neurons tested, this current was mimicked by the β-adrenergic agonist isoprenaline.

DISCUSSION

The main finding of this study is that noradrenaline dramatically increases inhibitory synaptic activity in both cholinergic- and non-cholinergic-type medial septal/diagonal band neurons; this increase in inhibitory activity results from a direct α_1-mediated excitation of GABAergic neurons present within the MSDB. Another significant finding of this study is that the NA-excited MSDB neurons belong to a subpopulation of GABAergic neurons which project to the hippocampus; NA effects in the MSDB are therefore likely to have a profound effect on septal as well as hippocampal function.

NA excites GABAergic neurons in the MSDB brain slice preparation

In the present study, bath-applied NA increased the frequency of inhibitory synaptic activity in both the cholinergic- and non-cholinergic-type (presumably, GABA-ergic) MSDB neurons. It was concluded that the NA-induced changes in synaptic activity result from an activation of GABAergic neurons located within the MSDB (some of which are quiescent under control conditions, as indicated by direct extracellular and intracellular recordings from NA-responsive neurons). The following observations support this conclusion: (1) the NA-induced increase in synaptic activity was blocked by bicuculline, a $GABA_A$ antagonist; (2) the change in the amplitude and polarity of the NA-induced synaptic currents was consistent with changes in the E_{Cl} – thus, in recordings with gluconate-containing electrodes (calculated $E_{Cl} = -80$ mV), the amplitude of the NA-induced synaptic currents decreased at more negative holding potentials and reversed polarity between −80 and −100 mV, whereas with chloride-containing electrodes (calculated $E_{Cl} = 1{\cdot}5$ mV), the IPSCs were reversed in polarity and showed an increase in amplitude at more negative holding potentials; (3) NA-induced IPSCs were blocked by tetrodotoxin (a fast, sodium channel blocker); (4) NA induced a similar increase in IPSCs in slices in which the MSDB had been surgically isolated from all neighbouring brain structures such as the lateral septum; (5) and in voltage-clamp recordings, GABA-type neurons that were directly excited by NA in a tetrodotoxin-insensitive manner were found within the MSDB complex.

A NA-induced excitation of GABAergic neurons has been reported in other areas of the brain such as the dentate gyrus (Rose & Pang, 1989) and the cerebellum (Llano & Gerschenfeld, 1993). Within the MSDB the GABAergic neurons excited by NA could belong to the subpopulation of GABAergic neurons that contains the Ca^{2+}-binding protein parvalbumin and projects to the hippocampus (Freund, 1989) and/or to the second subpopulation that does not project outside of the nucleus. Since we observed a profound increase in local synaptic activity following bath application of NA, we concluded that the NA-activated MSDB GABA-ergic neurons make numerous local circuit connections. Anatomically, the presence of such local connections has been much speculated about as parvalbumin-immunoreactivity terminals have been found around both parvalbumin-positive and parvalbumin-negative MSDB structures (Leranth *et al.* 1992; Gao, Hornung & Fritschy, 1995). The results of the

present study and a previous study on the effects of serotonin on MSDB neurons (Alreja, 1996) provide strong evidence for the presence of such local connections.

NA excites septohippocampal neurons with fast conducting fibres

Interestingly, in addition to making local synaptic connections, the NA-activated MSDB neurons also project to the hippocampus. This conclusion is based on the results of antidromic studies, which indicate that NA excites septohippocampal neurons, and is consistent with the anatomical demonstration of direct synaptic contacts between noradrenaline terminals and septohippocampal neurons (Milner *et al.* 1995). Additionally, the evidence presented in this study indicates that the NA-excited septohippocampal neurons are most likely GABAergic in nature. This is suggested by the short antidromic activation latencies of NA-responsive neurons and thus higher mean conduction velocities ($1{\cdot}74 \pm 0{\cdot}11$ m s^{-1}). Conduction velocities in this range have previously been reported to represent the thickly myelinated, faster conducting GABAergic fibres (Freund, 1989; Miller & Freedman, 1993). In contrast, the unmyelinated or lightly myelinated cholinergic SHNs have much slower conducting fibres ($< 0{\cdot}3$ m s^{-1}). The conclusion that the NA-excited SHNs are indeed GABAergic in nature is also supported by the consistent increase in GABAergic synaptic activity that was observed following bath application of NA. The strong excitation of GABAergic SHNs by NA is likely to have a significant effect on hippocampal function as the GABAergic projection neurons of the MSDB innervate almost every type of hippocampal interneuron (Freund & Antal, 1988). A NA-induced excitation of septohippocampal GABAergic neurons would therefore inhibit a large number of hippocampal GABAergic neurons and thereby reduce both the feed-back and feed-forward type of local hippocampal inhibition to a minimal level (Freund & Antal, 1988). The resultant increase in excitability, amongst other effects, could promote induction of long-term potentiation (LTP) as LTP can be preferentially induced when the cells are maximally stimulated (Pavlides, Greenstein, Grudman & Winson, 1988). Therefore, the results of the present study suggest that NA via its actions on MSDB GABAergic neurons would not only have profound local effects (by virtue of a large number of local circuit connections) but would also strongly influence hippocampal function.

α_1-Adrenoceptors mediate NA-induced excitatory responses of septohippocampal GABAergic neurons

In the present study, the α_1-receptor subtype was found to mediate the excitatory responses to NA in MSDB GABAergic neurons. These findings are consistent both with the moderate levels of [^{3}H]prazosin binding that have been reported in the rat MSDB (Palacios *et al.* 1987) and with receptor mRNA studies that demonstrate the presence of both $\alpha_{1A/D}$ and α_{1B} receptor mRNA in the rat MSDB (Pieribone *et al.* 1994). In the present study, the α_1-antagonists prazosin and WB-4101 blocked extracellularly recorded excitatory responses to NA with mean pA_2 values of $8{\cdot}53 \pm 0{\cdot}12$ and $8{\cdot}18 \pm 0{\cdot}34$, respectively, which are close to those reported in binding studies in the cerebral cortex (Morrow & Creese, 1986). Consistent with the presence of α_1-receptors, the α_1-agonist phenylephrine also mimicked the excitatory responses of NA in septohippocampal neurons.

Similarly, in whole-cell recordings, phenylephrine mimicked the actions of NA and increased the frequency of bicuculline- and TTX-sensitive IPSCs. Also in consonance with the reported partial agonistic properties of phenylephrine, IPSCs recorded following application of near-maximal concentrations of PE or NA had a statistically similar amplitude but different frequency distribution as fewer events were recorded with PE.

Additionally, in a subpopulation of GABA-type MSDB neurons with short-duration spikes, NA-induced an inward current that was mimicked by PE and blocked by the α-antagonist prazosin with a pA_2 of 8·7, confirming the presence of direct, α_1-mediated excitatory responses to NA in the MSDB. The finding that GABAergic neurons in the MSDB are excited by NA and PE but also receive more inhibitory synaptic inputs is functionally paradoxical. However, the net effect on GABAergic neurons is still an excitation (as evidenced by studies with gluconate-containing patch electrodes and by extracellular recordings). Therefore, it appears that the local GABAergic circuitry serves primarily to limit the NA-induced excitation of MSDB GABAergic neurons.

Implications of the findings

In addition to its impact on septohippocampal circuitry, the profound excitatory effecs of NA on MSDB neurons reported in this study could in part explain the increased glucose utilization that occurs in the MSDB region during opiate withdrawal (Kimes & London, 1988). During opiate withdrawal NA release in the MSDB would be enhanced, as the activity of noradrenergic neurons of the locus coeruleus (which provide the major noradrenergic input to the MSDB) is significantly increased during opiate withdrawal (Rasmussen & Aghajanian, 1989). Of course, a direct involvement of the MSDB in the opioid abstinence syndrome may also contribute to the increased glucose utilization in the MSDB during the abstinence syndrome (Kosten, Lee & Alreja, 1995). In contrast to their increased activity during opiate withdrawal, the pacemaker neurons of the locus coeruleus stop firing during rapid eye movement (REM) sleep; this would lead to an opposite change, i.e. a decrease in basal release of NA in the MSDB. Interestingly, a marked increase in hippocampal acetylcholine release has been reported during REM sleep (Marrosu *et al.* 1995), a finding that would be predicted by the results of the present study, as a decrease in NA-induced excitation of GABAergic neurons would lead to a disinhibition of septal cholinergic neurons.

In conclusion, this study describes novel α_1-adrenoceptor-mediated excitatory actions of NA on septohippocampal

GABAergic neurons which by virtue of local connections as well as hippocampal projections would influence both septal and hippocampal functioning.

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