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COMBINED ANTAGONISM OF AMINERGIC EXCITATORY AND AMINO ACID INHIBITORY RECEPTORS IN THE XII NUCLEUS ABOLISHES REM SLEEP-LIKE DEPRESSION OF HYPOGLOSSAL MOTONEURONAL ACTIVITY

V. FENIK, R.O. DAVIES AND L. KUBIN[1]

Department of Animal Biology, School of Veterinary Medicine and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA 19104-6046, USA

INTRODUCTION

During REM sleep, many central neurons increase their activity while somatosensory transmission and motor tone are suppressed (4, 6, 31, 35). Four distinct neurochemical mechanisms acting at the motoneuronal level have been proposed to cause the motor atonia of REM sleep. Two rely on active, state-specific inhibition mediated by glycine and/or $GABA_A$ receptors (7, 26); two are disfacilitatory and based on a REM sleep-related withdrawal of motoneuronal excitation mediated by serotonin (5-HT) and norepinephrine (17, 22).

In hypoglossal (XII) motoneurons, the subject of this study, strychnine-sensitive (glycinergic), hyperpolarizing potentials occur following pontine microinjections of a cholinergic agonist, carbachol, that trigger REM sleep-like postural atonia (13, 43). However, microinjections into the XII nucleus of either strychnine or bicuculline, a $GABA_A$ receptor antagonist, had little effect on the suppression of XII nerve activity elicited by pontine carbachol in decerebrate cats (18), and a similar observation was made in chronically instrumented, naturally sleeping rats (27). These results show that, while an amino acid-mediated inhibition increases in XII motoneurons during REM sleep, its effect makes a small contribution to the suppression of motoneuronal activity.

The evidence for a REM sleep-related withdrawal of serotonergic and noradrenergic excitation from XII motoneurons is based on the following observations: i) both serotonergic and noradrenergic neurons project to the XII nucleus (1, 23), and both amines excite XII motoneurons (9, 20); ii) serotonergic and noradrenergic brainstem neurons are silenced during both natural REM sleep (5, 24, 33, 36) and the cholinergically-induced REM sleep-like state (12, 32, 40); and iii) the extracellular levels of both 5-HT and norepinephrine are reduced in the XII nucleus region during REM sleep-like atonia (19, 22). However, infusion of 5-HT into the XII nucleus only partially blunted the carbachol-induced suppression of XII nerve activity in decerebrate cats (21) and that occurring during natural REM sleep in rats (14).

[1] Corresponding Author: Leszek Kubin, Ph.D., Department of Animal Biology 209E/VET, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104-6046, USA. Tel. 001-215-898-1893; Fax. 001-215-573-5186; e=mail: lkubin@vet.upenn.edu.

Together, these results suggest that multiple mechanisms converge on motoneurons to suppress their activity during REM sleep. The goal of this study was to determine whether the REM sleep-like suppression of XII motoneuronal activity can be fully accounted for by the combined actions of 5-HT, norepinephrine, GABA and glycine. To test this hypothesis, we used urethane-anesthetized rats in which REM sleep-like episodes are elicited by carbachol microinjections into a distinct site within the dorsal pontine tegmentum. These episodes can be triggered repeatedly and comprise events typical of REM sleep, such as cortical activation, hippocampal theta rhythm, silencing of pontine noradrenergic neurons, suppression of XII nerve activity and slowing of the respiratory rate (12, 16). Using this model, we found that a combined antagonism of serotonergic, noradrenergic, $GABA_A$ and glycinergic receptors in the XII nucleus region eliminates the depressant effect of pontine carbachol injections on XII motoneuronal activity. A preliminary report has been published (10).

METHODS

Animal preparation and monitoring.

Experiments were performed on six adult male Sprague-Dawley rats weighing 408 g ± 9.5 (SE). The procedures for anesthesia, surgery and recording followed the guidelines of the Institute for Laboratory Animal Research, and were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

The animals were anesthetized with isoflurane (2%) followed by urethane (1 g•kg^{-1}, i.p., supplemented by 50 mg i.v. injections as needed). The trachea was intubated, and a femoral artery and vein were cannulated for arterial blood pressure monitoring and fluid injections, respectively. The right XII nerve was dissected and placed in a cuff-type recording electrode (11). Both cervical vagal trunks were cut to enhance XII nerve activity and make it independent of lung volume feedback. The animal was placed in a stereotaxic head holder, two openings made in the right parietal bone, and the dura removed for inserting a carbachol-containing pipette and hippocampal recording electrode. The dorsal surface of the caudal medulla was exposed to insert a microinjection pipette into the XII nucleus.

For monitoring the cortical EEG, a screw was attached to the frontal bone 2 mm anterior and 2 mm to the right, and a second one to the parietal bone 3 mm posterior and 2 mm to the left, of the bregma. To record hippocampal activity, two insulated platinum wires (50/100 μm bare/coated diameter, A-M Systems) with tips separated by 0.8 mm were placed 3.7 mm posterior to, and 2.2 mm to the right of, the bregma and 2.4 mm below the cortical surface. The final position of this electrode was adjusted to maximize the amplitude of the theta rhythm elicited by a foot pinch.

The animals were paralyzed (pancuronium bromide, 2 mg•kg^{-1} i.v., supplemented with 1 mg•kg^{-1} injections as needed) and artificially ventilated with an air-oxygen mixture (O_2 concentration 30-60%) at 50-70 lung inflations/min. A regular central respiratory rhythm, steady XII nerve electroneurogram and blood pressure, and only transient activations of cortical and hippocampal activities in response to pinch indicated that the animal was adequately anesthetized. All recordings were obtained from animals with a systolic blood pressure above 85 mmHg and rectal temperature of 36-37 °C. The end-expiratory CO_2 was continuously measured (Columbus Instruments capnograph). We intended to maintain CO_2 levels constant throughout each experiment. This was successful in three rats, but in the other three, XII nerve activity was abolished or nearly abolished following antagonist injections into the XII nucleus. In these rats, to restore XII nerve activity to a level suitable for reliable measurements, ventilation was reduced after the first post-antagonist test with carbachol. This interrupted the continuity of our observations of changes in basal XII

nerve activity induced by the antagonists, but did not affect our ability to observe the effect of subsequent carbachol injections. The CO_2 was 5.6% ± 0.3 (SE) in the three rats in which it was kept constant, and in the other three it was increased during the course of the experiment to 7.4% ± 0.2.

Hippocampal (bandwidth 3-8 Hz), cortical (1-100 Hz), and XII nerve (30-2500 Hz) activities were recorded with AC amplifiers (N101, NeuroLog). XII nerve activity was full-wave rectified and a moving average derived (time constant 100 ms; MA-821 RSP; CWE, Inc.). All neural signals, event markers, blood pressure, tracheal pressure and end-expiratory CO_2 were monitored on a chart recorder (TA-11; Gould Instruments), and recorded on a 16-channel digital tape recorder (C-DAT; Cygnus Technology).

Drug solutions and microinjections.

Methysergide maleate salt (5-HT receptor antagonist), strychnine nitrate salt (glycine receptor antagonist), (-)-bicuculline methiodide ($GABA_A$ receptor antagonist), and carbamylcholine chloride (carbachol, cholinergic receptor agonist) were obtained from Sigma (St. Louis, MI), and 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl) piperazine hydrochloride (prazosin, α_1-adrenoceptor antagonist) from RBI (Natick, MA). The stock solution of prazosin was made in distilled water and all other drugs in 0.9% NaCl. The final antagonist solution (antagonist mix) was prepared in 0.9% NaCl from aliquots of frozen stock solutions or freshly dissolved drugs about one hour before injections and was composed of (in mM): methysergide 1.0, prazosin 0.2, bicuculline 1.0, and strychnine 1.0. At these concentrations, the antagonist mix blocked the effects of the corresponding agonists when they were injected into the XII nucleus at the following concentrations: 2 mM phenylephrine, 5 mM 5-HT, 20 mM muscimol and 5 mM glycine (data not shown).

For the carbachol injections, a glass pipette (tip diameter 20-30 μm) filled with 10 mM carbachol and 2% Pontamine sky blue dye (ICN) in 0.9% saline was inserted into the dorsomedial pontine reticular formation using surface landmarks established in earlier studies (12, 41). Ten nanoliters of carbachol (18.3 ng) were injected over 10-20 s by applying pressure to the fluid in the pipette while monitoring the movement of the meniscus with a calibrated microscope.

For injections into the XII nucleus, a glass pipette with the tip beveled to an external diameter of 22-28 μm was filled with the antagonist mix and inserted into the right XII nucleus 0.3 mm lateral to the midline and 1.15 mm below the dorsal medullary surface at three locations: 0.5 mm caudal, 0.15 mm rostral and 0.8 mm rostral to the calamus scriptorius. Three successive injections of the antagonist mix, 40 nl each, were placed in the XII nucleus over 8.2 min ± 0.9 (SE). The location and volume of the injections were selected to ensure that the drugs spread throughout the XII nucleus. Specifically, with the available extracellular space in the CNS estimated to be ~ 21% of the total volume of the tissue (28), each 40 nl injections would initially fill a sphere having a diameter of 0.71 mm, which is close to the medio-lateral dimension of the XII nucleus in the rat (3). With the 0.65 mm rostro-caudal spacing of successive injections, these three injections delivered drugs to the XII nucleus along its entire rostro-caudal extent.

Experimental protocol and data analysis.

In each experiment, multiple pontine carbachol injections were performed at a minimum of 30 min intervals; at this repetition rate, the responses occur without adaptation (12, 41). At least two injections (10 nl each) were performed at the beginning of each experiment to verify that the response had a REM sleep-like pattern and to ensure repeatability. Once the control response was established, the pipette containing the antagonist mix was inserted into the XII nucleus and three injections made at the three rostro-caudal locations described above. After the last antagonist injection, carbachol was injected into the pons to elicit the first post-antagonist response. Additional carbachol injections were then made during the subsequent three hours to observe the time-course of the effects of antagonists on XII nerve activity and its response to pontine carbachol.

Changes in XII nerve activity were assessed from the moving average of the signal by measuring the difference between its peak during the inspiratory phase of the respiratory cycle and an expiratory period when no activity was present. The central respiratory rate was determined from the record of XII nerve activity. When XII nerve activity was transiently abolished during some responses to carbachol, the respiratory rate was determined from the last respiratory cycle prior to

the disappearance of the activity. The latencies and durations of the responses were measured from the onset of the carbachol injection to the start and end of the changes in hippocampal activity. The time of the maximal response was identified from the changes in XII nerve activity and respiratory rate. The average measures for each response were obtained from 30 s segments of records prior to, centered around the point of maximal effect, and after the recovery from the effect of carbachol. To track the changes caused by antagonist injections into the XII nucleus, the level of XII nerve activity during the course of each experiment was normalized by the level of activity just prior to the control response to carbachol. The magnitudes of the effect of carbachol on XII nerve activity were expressed relative to the activity level just prior to each carbachol injection.

After verification that the data were normally distributed, paired, two-tailed Student's t-test or two way ANOVA with Bonferroni's correction for multiple comparison were used for statistical analyses (SigmaStat, Jandel). Differences were considered significant when $p < 0.05$. The variability of the means is characterized by the standard error (SE) throughout the report.

Histology.

At the end of selected experiments, microinjection sites in the XII nucleus were iontophoretically marked with Pontamine blue dye. All animals were given urethane (2 g•kg^{-1} i.v.) and decapitated. The brainstem was removed, fixed in 10% phosphate buffered formalin and cut into 100 µm sections in the coronal plane for the pons, and sagittal or coronal plane for the medulla. Sections containing the Pontamine blue dye were serially mounted and stained with neutral red.

RESULTS

Under the control conditions, pontine carbachol injection triggered REM sleep-like episodes characterized by the appearance of hippocampal theta rhythm (3-4 Hz), a profound suppression of XII nerve activity and a decreased respiratory rate, as described previously (12, 16). Figure 1A shows the pontine carbachol injection sites

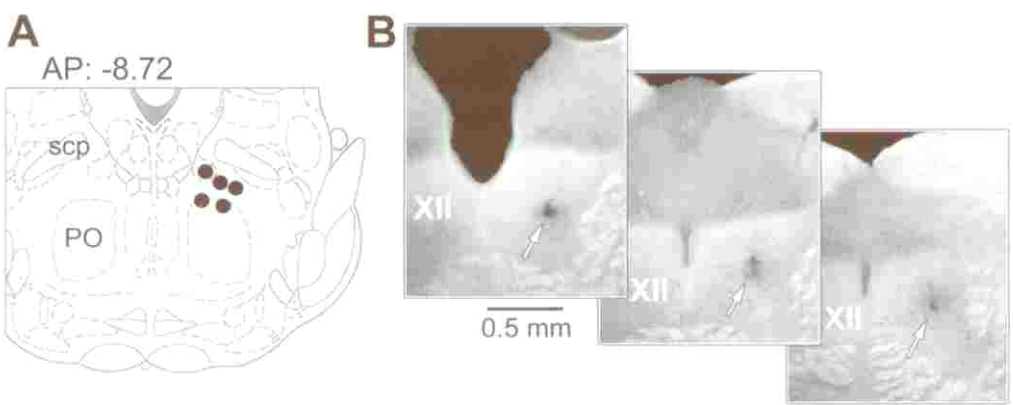

Fig. 1. - *Localization of pontine carbachol injections (A), and an example of the locations of the three antagonist injections in the XII nucleus (B).*

Circles in A show the centers of carbachol injections in five rats superimposed on the corresponding standard section of the pons (30). B: dark field images of the coronal sections through the dorsomedial medulla taken from three rostro-caudal levels spaced 0.65 mm apart. Arrows point to iontophoretically marked centers of antagonist microinjection sites in the XII nucleus. Abbreviations: PO - nucleus pontis oralis; scp - superior cerebellar peduncle; XII - hypoglossal nucleus.

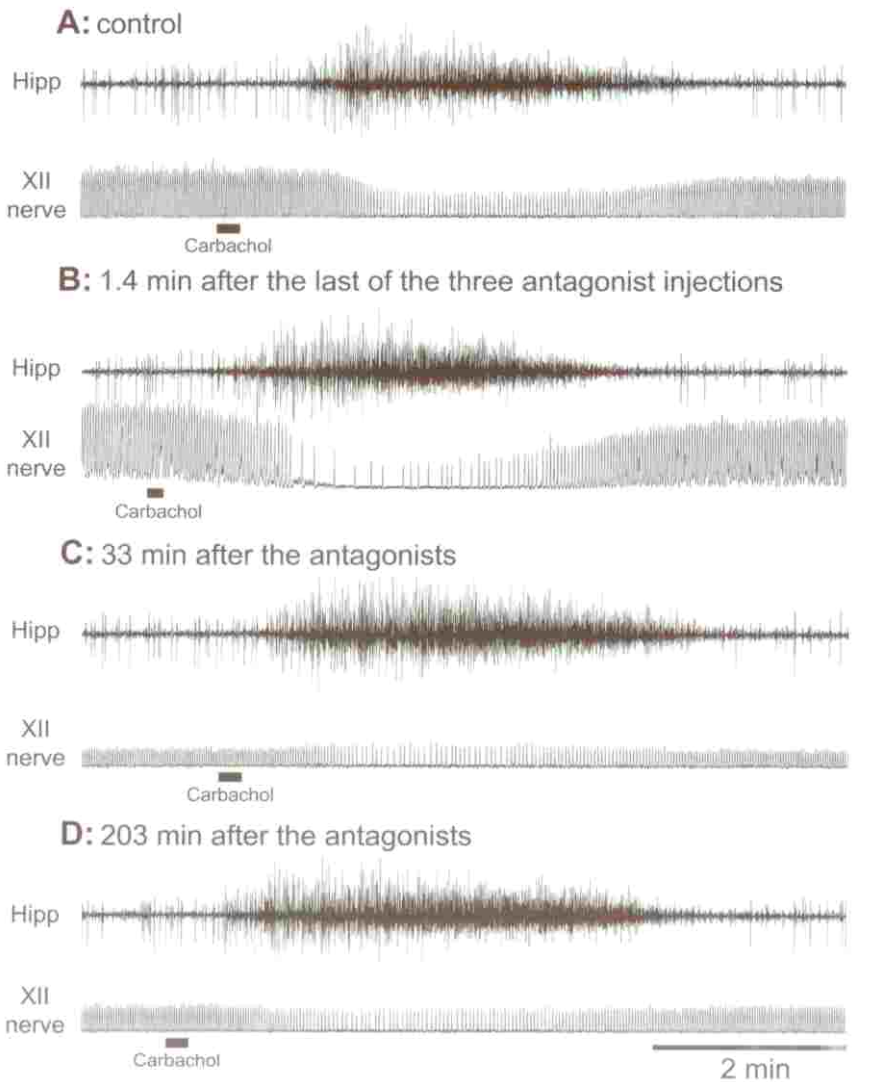

Fig. 2. - *The antagonist mix containing methysergide, prazosin, bicuculline and strychnine abolishes the REM sleep-like depression of XII nerve activity elicited by pontine carbachol ~ 30 min after the antagonists.*

In each panel, the top trace shows hippocampal activity (Hipp) and the bottom trace the moving average of XII nerve activity, both recorded at the same gains in all four panels; 10 nl carbachol injections made at the markers. A: the control REM sleep-like depression of XII nerve activity. B: Response to carbachol elicited just after the antagonists; there was a marked depression of XII nerve activity despite the basal activity level being strongly enhanced by the antagonists. C: 33 min after the antagonists, the pre-carbachol level of XII nerve activity was reduced (compare to XII nerve activity prior to carbachol in A). No depression occurred in response to carbachol; instead, there was a small increase at the time when hippocampal activation and slowing of the respiratory rate were maximal. D: 203 min after the antagonists; the pre-carbachol level of XII nerve activity increased compared to that in C, and it was then depressed during the response to pontine carbachol, both indicating a partial recovery from the abolition of the REM sleep-like depression illustrated in C.

for 5 of the 6 experiments (one site was not determined, but the responses to carbachol had all the features of a typical REM sleep-like effect). An example of a typical response to pontine carbachol is shown in Figure 2A. Parallel to hippocampal activation, an increase in the high-frequency components of the cortical EEG occurred during all responses; for simplicity, these cortical changes are not illustrated.

The control responses were elicited with a mean latency of 29.0 s ± 6.0 and had a mean duration of 229 s ± 23 (n = 6). The maximal suppression of XII nerve activity was to 18.1% ± 7.2 of its pre-carbachol level ($p < 0.001$). The respiratory rate decreased during these responses from 45.6 min^{-1} ± 2.0 before, to 24.5 min^{-1} ± 4.2 ($p < 0.001$). The magnitudes of individual and average XII nerve activity and respiratory rate responses to carbachol under the control conditions are shown in the left panels of Figure 3B and C.

In each of the six rats, three injections of the antagonist mix were made into the XII nucleus 50 min ± 11 after the control response to pontine carbachol. The three injection sites were distributed evenly along the rostro-caudal span of the nucleus and centrally located at each rostro-caudal level (Fig. 1B). The injections caused no obvious changes in respiratory rate or arterial blood pressure, but elicited a large increase of XII nerve activity. The increase occurred incrementally with each injection, reached a maximum of 197% ± 40 of the control level of XII nerve activity about 1 min after the last injection and then the activity declined over the next 20-30 min. In three rats, XII nerve activity disappeared or nearly disappeared at this time, necessitating a reduction of ventilation to restore activity. Ventilation was then left unchanged for the rest of these experiments. In the three rats in which ventilation and CO_2 level remained unchanged throughout the experiments, the lowest level of XII nerve activity was 12.4% ± 1.9 of that prior to the antagonists. Subsequently, the activity gradually increased, and at 180 min after the antagonists reached 43.7% ± 7.9 of the pre-antagonist level. Figure 3A shows the time-course of the antagonists effect on the spontaneous XII nerve activity in the three rats.

In four rats, the first post-antagonist tests with pontine carbachol were conducted 1.2-2.9 min after the last of the three antagonist injections. The resulting REM sleep-like episodes had a mean latency of 25.5 s ± 7.6 and a duration of 261 s ± 53 (not different from the pre-antagonist values). One example is shown in Figure 2B. During these responses, XII nerve activity was suppressed to 21.1% ± 9.2 ($p < 0.01$) of the pre-carbachol level (Fig. 3B). This was not statistically different from the responses prior to the antagonists ($F_{1,1,3} = 1.3$; $p = 0.33$ two-way repeated measures ANOVA). The respiratory rate prior to these carbachol injections was slightly lower than that prior to the pre-antagonist responses to carbachol, 40.2 min^{-1} ± 1.9 *vs.* 45.6 min^{-1} ± 2.0 ($p < 0.05$). Nevertheless, its carbachol-induced decrease to 17.1 min^{-1} ± 4.7 was significant ($p < 0.01$), and not significantly different from the pre-antagonist responses ($F_{1,1,3} = 0.67$; $p = 0.47$).

The next set of post-antagonist tests with carbachol was conducted in all six rats 30-60 min after the last of the antagonist injections. The responses to carbachol had a mean latency of 33.3 s ± 6.0 and a duration of 242 s ± 46 (not different from the control, pre-antagonist values). At this time, the pre-carbachol level of XII nerve activity was greatly reduced, as described above (Fig. 3A). The response to carba-

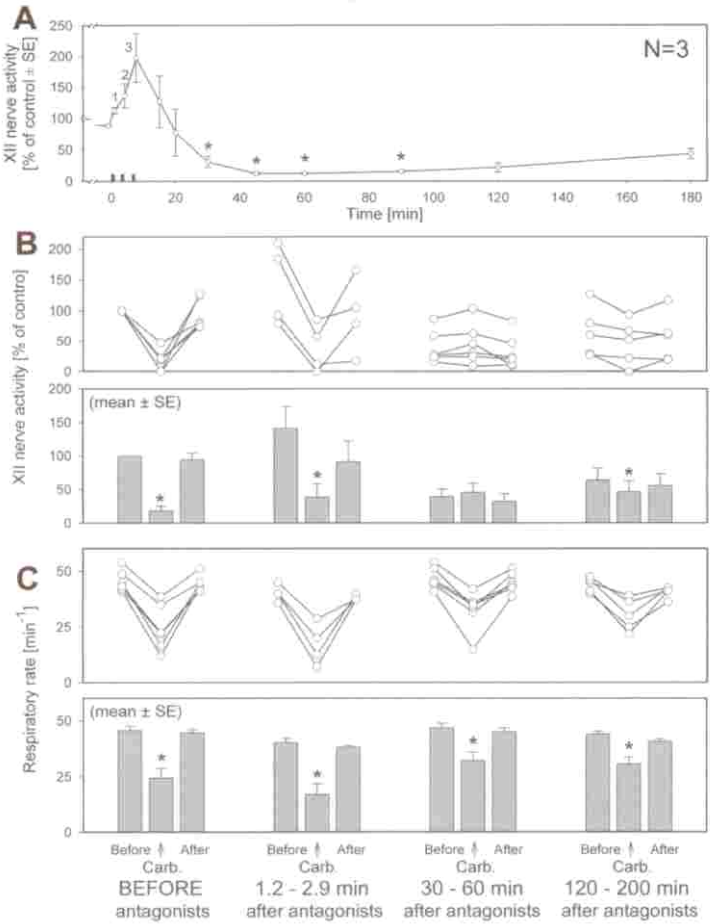

Fig. 3. - *The time course of the changes in spontaneous XII nerve activity following microinjections of the antagonists into the XII nucleus (A), and the individual and average effects of the antagonists on the carbachol-induced depression of XII nerve activity (B) and slowing of the respiratory rate (C) at successive stages of the protocol.*

A: the average changes in XII nerve activity during a 3 h period after three successive injections of the antagonist mix into the XII nucleus in three rats. The antagonists were injected at three sites in the XII nucleus between time 0 and the point numbered 3 (points 1-3 show activity levels measured 1 min after each of the three injections). After an initial increase, XII nerve activity declined over a period of 20-30 min to a minimum level that was significantly lower than that prior to the antagonists (* - $p < 0.05$). Then, a recovery to nearly 50% of the pre-antagonist level occurred over the next 2 h. B: individual (top) and average (bottom) responses of the XII nerve to pontine carbachol. Each data set shows the level of XII nerve activity just before, at the peak of the response, and just after the response to carbachol (* - different from the pre-carbachol level at $p < 0.05$). The four data sets illustrate the effects of carbachol under control conditions and then during three periods after the antagonists, as indicated at the bottom of the figure. XII nerve activity was significantly depressed by pontine carbachol at all stages of the experiments except when carbachol injections were made 30-60 min after the antagonists. During this period, instead of a decrease, a small increase of XII nerve activity occurred in four out of six rats. XII nerve activity in A and B is normalized by the activity level prior to the pre-antagonist response to carbachol. C: Changes in the respiratory rate elicited by pontine carbachol at successive stages of the protocol shown using the same format as in B. The baseline respiratory rate and its carbachol-induced changes were well maintained at all times after the antagonists.

chol again included the characteristic hippocampal and cortical activations, and the respiratory rate decreased from 46.8 min^{-1} ± 2.0 to 32.2 min^{-1} ± 3.7 ($p < 0.01$), which was not different from the responses elicited prior to the antagonists ($F_{1,1,5} = 3.0$; $p = 0.15$). However, during these responses, XII nerve activity tended to increase, rather than decrease. Figure 2C shows one example of this effect. The average increase was 11% ± 15 ($n = 6$, $p = 0.18$) above the pre-carbachol level, with clear increases observed in four, no change in one, and a small decrease in one experiment (Fig. 3B). This absence of depression of XII nerve activity was statistically different from the depressant effects observed before the antagonists ($F_{1,1,5} = 150$; $p < 0.001$).

Following the carbachol injections placed with longer delays after the antagonists, the depressant effect on XII nerve activity partially recovered (Figs. 2D, 3). In the tests performed in five rats 120-200 min after the antagonists, XII nerve activity was suppressed by carbachol to 65.3% ± 16 of the pre-carbachol level ($p < 0.05$). The latencies and durations of the responses were not different from those prior to the antagonists, 28.8 s ± 3.0 and 198 s ± 32, respectively, and the respiratory rate decreased from 44.0 min^{-1} ± 1.4 to 31.8 min^{-1} ± 3.8 ($p < 0.05$) (Fig. 3B and C, right panels).

DISCUSSION

Our main finding is that microinjection into the XII nucleus of antagonists of four receptor systems eliminates the depressant, REM sleep-like, effect of pontine carbachol on the activity of XII motoneurons. The antagonistic effect was selective in that the depression of XII nerve activity was abolished, but other REM sleep-like changes, such as slowing of the respiratory rate and activation of the cortical and hippocampal activities, remained intact. This indicates that, at least in the carbachol model, the REM sleep-like suppression of XII motoneuronal activity can be fully accounted for by some or all of the following: a withdrawal of serotonergic and noradrenergic excitation and increased inhibition mediated by GABA and glycine. This finding is an important first step towards an assessment of the relative contributions of these four distinct receptor systems to the REM sleep-like suppression of motoneuronal activity.

Urethane-anesthetized rat as a model of the neural phenomena of REM sleep.

In urethane-anesthetized rats, carbachol injections into a discrete site within the dorsomedial pontine tegmentum elicit ~ 4 min responses that include the activation of cortical EEG, the appearance of rhythmic theta-like activity in the hippocampus, silencing of pontine noradrenergic neurons, and a profound suppression of XII nerve activity (12, 16). These responses are similar to the episodes of natural REM sleep and are triggered from a site similar to that identified in cats as most effective for triggering of REM sleep-like state (37, 42). Thus, under urethane anesthesia, properly placed carbachol injections can activate an important subset of the same neural events as those activated during natural REM sleep. This said, we are aware that this is a reduced model of the corresponding behavioral state, and additional mechanisms may influence the activity of XII motoneurons during REM sleep in behaving animals.

Rationale for the composition of the antagonist mix.

Evidence from both naturally sleeping animals and carbachol models implicates each of the four neurochemically distinct mechanisms studied here in the depression of motoneuronal activity during REM sleep. Based on the presence in motoneurons of strychnine-sensitive inhibitory postsynaptic potentials and increased extracellular levels of glycine and GABA in motor nuclei, it was proposed that active inhibition causes the depression of motoneuronal activity during REM sleep (13, 15, 25, 43). Other studies demonstrated that the levels of 5-HT and norepinephrine are reduced in motor nuclei (19, 22), and that aminergic brainstem cells that project to motor nuclei are silenced during the REM sleep-like conditions (12, 40). Attempts were previously made to test whether, by acting on motoneurons, a single mechanism is responsible for the atonia of REM sleep. The findings that glycinergic, GABAergic or serotonergic drugs alone could not fully account for the atonia (14, 18, 21, 27, 34) suggested that the atonia of REM sleep is mediated by convergent effects of multiple neurotransmitter systems. If so, antagonism of all relevant motoneuronal receptors should eliminate those inputs to XII motoneurons whose magnitude changes during REM sleep. The design of our study was guided by the findings that aminergic excitatory effects in XII motoneurons are mediated by 5-HT_{2A} and α_{1B} adrenergic receptors, which are blocked by methysergide and prazosin, respectively (9, 38, 39, 44). The concentrations of these and the remaining two antagonists were based on our earlier experience with these drugs (9, 18), and verification that the antagonist mix used in this study blocked the effects on XII nerve activity of the corresponding agonists injected into the XII nucleus *in vivo*.

Abolition of the REM sleep-like depression of XII nerve activity and its timing.

With the volumes of the antagonist injections theoretically sufficient to spread over the entire XII nucleus, we expected the depressant effect of carbachol to be eliminated almost immediately after the antagonists. However, the depression of XII nerve activity was well preserved during the tests performed just after the last antagonist injection. This suggests that antagonistic actions exerted solely on receptors located within the XII nucleus are not sufficient to block the effect of pontine carbachol and that spread of drugs beyond the XII nucleus was necessary.

Injections of the antagonist mix initially caused a nearly two-fold increase of XII nerve activity. This was followed by a decline to a level substantially lower than that prior to the antagonists. One explanation for such a biphasic change would be that the disinhibitory effect of strychnine and/or bicuculline gradually weakened, while the antagonism of the endogenous excitation mediated by 5-HT and/or norepinephrine remained strong. If so, the abolition of the atonia with some delay after the antagonists suggests that the combined antagonism of serotonergic and noradrenergic excitation plays a major role in eliminating the carbachol-induced changes in XII nerve activity. However, by itself, bicuculline injected into the XII nucleus causes a delayed decrease of XII nerve activity, presumably by its actions exerted in the reticular formation adjacent to the XII nucleus (18). Accordingly, 30-60 min after the antagonists, the substantially reduced level of XII nerve activity probably reflected a net effect of disinhi-

bition and disfacilitation within the nucleus, and additional actions exerted in the surrounding reticular formation. At this time, the carbachol-induced depression of XII nerve activity was abolished, but the design of our study did not allow us to determine when the abolition first occurred because the minimum interval between successive carbachol injections was longer than 30 min. Carbachol had a significant depressant effect just after the antagonists were injected when their concentrations in the XII nucleus were highest. The depressant effect of carbachol was abolished during the subsequent 10-40 min, and then the effect partially recovered 1-2 hours later. This time course is compatible with a genuine antagonistic action of the drugs and their gradual diffusion and removal, rather than with their unspecific or toxic effect.

The diffusion of drugs beyond the XII nucleus must have been relatively limited because the antagonists did not elicit any obvious changes in respiratory rate or blood pressure. Nevertheless, the drugs probably spread to extranuclear dendrites of XII motoneurons (3) and other neurons around the XII nucleus. Our present data do not allow us to determine the extent of effective diffusion or assess the relative contribution of extranuclear drug actions. It is, however, noteworthy that the distal dendrites of XII motoneurons are closely apposed by both serotonergic and noradrenergic terminals and have 5-HT_{2A} receptors (1, 2, 8).

In four out of the six rats, a small but clear increase of XII nerve activity, rather than a depression, occurred following pontine carbachol injections made at the time when the antagonists maximally reduced the baseline XII nerve activity. This effect could be due to an unmasking of excitatory effects of pontine carbachol on motoneurons at the time when the depressant effects were abolished. For example, similar to natural REM sleep (29), some premotor neurons that relay inspiratory drive to XII motoneurons increase their activity during the REM sleep-like atonia in urethane-anesthetized rats (41).

In summary, we found that microinjections into the XII nucleus of the antagonists of four receptor systems, serotonergic, noradrenergic, $GABA_A$ and glycinergic, are sufficient to abolish the REM sleep-like depression of XII motoneuronal activity. This finding delineates a limited set of neurotransmitter receptors that may mediate the atonia in XII motoneurons. All four antagonists may not be needed to achieve this effect, and those that are necessary may need to act both within and adjacent to the XII nucleus. These findings provide the basis for a further spatial localization and pharmacological characterization of the mechanisms that mediate the REM sleep atonia in XII and other motoneurons.

SUMMARY

It is hypothesized that the suppression of motor activity (atonia) that occurs during REM sleep is caused by the combined inhibition of motoneurons by glycine or GABA and withdrawal of excitation mediated by serotonin and norepinephrine. However, it is not known whether these mechanisms can fully account for the atonia. In urethane-anesthetized, paralyzed and artificially ventilated rats, REM

sleep-like episodes can be repeatedly elicited by microinjections of a cholinergic agonist, carbachol, into the dorsomedial pons. We used this model to determine whether microinjections of a combination of antagonists of serotonergic, adrenergic, $GABA_A$ and glycinergic receptors (methysergide, prazosin, bicuculline and strychnine) into the XII nucleus can abolish the carbachol-induced depression of XII motoneuronal activity. REM sleep-like episodes were elicited prior to, and at different times after, antagonist microinjections. In all six rats studied, the depression of XII motoneuronal activity did not occur when tested 30-60 min after the antagonists, whereas other characteristic features of the response (latency, duration, the appearance of hippocampal theta rhythm, activation of the cortical EEG, slowing of the respiratory rate) remained intact. The carbachol-induced depression partially recovered after 2-3 hours. We conclude that the REM sleep-like depression of XII motoneuronal activity can be fully accounted for by all or some of the following mechanisms: a withdrawal of motoneuronal excitation mediated by norepinephrine and serotonin and increased inhibition mediated by GABA and glycine.

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