Serotonergic modulation of hippocampal pyramidal cells in euthermic, cold-acclimated, and hibernating hamsters

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Horrigan, D. J., B. A. Horwitz, and J. M. Horowitz. Serotonergic modulation of hippocampal pyramidal cells in euthermic, cold-acclimated, and hibernating hamsters. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1291-R1298, 1997.—Serotonergic fibers project to the hippocampus, a brain area previously shown to have distinctive changes in electroencephalograph (EEG) activity during entrance into and arousal from hibernation. The EEG activity is generated by pyramidal cells in both hibernating and nonhibernating species. Using the brain slice preparation, we characterized serotonergic responses of these CA1 pyramidal cells in euthermic, cold-acclimated, and hibernating Syrian hamsters. Stimulation of Shaffer collateral/commissural fibers evoked fast synaptic excitation of CA1 pyramidal cells, a response monitored by recording population spikes (the synchronous generation of action potentials). Neuroumodulation by serotonin (5-HT) decreased population spike amplitude by 54% in cold-acclimated animals, 80% in hibernating hamsters, and 63% in euthermic animals. The depression was significantly greater in slices from hibernators than from cold-acclimated animals. In slices from euthermic animals, changes in extracellular K+ concentration between 2.5 and 5.0 mM did not significantly alter serotonergic responses. The 5-HT1A agonist 8-hydroxy-2-(di-n-propylamino)tetrinal mimicked serotonergic inhibition in euthermic hamsters. Results show that 5-HT is a robust neuromodulator not only in euthermic animals but also in cold-acclimated and hibernating hamsters.

5-hydroxytriptamine; 8-hydroxy-2(di-n-propylamino)tetrinal; hippocampal slice; temperature

5-HT (5-hydroxytryptamine) appears to play a pivotal role in the central neural control of hibernation. Brain 5-HT levels increased their hibernation in a number of mammalian species, including the hedgehog (30), the ground squirrel (19), and the Syrian hamster (21). However, 5-HT levels in hibernating European hamsters were lower than in awake animals (8), an observation that may reflect either species or technique differences. A more direct measure of serotonergic activity is 5-HT turnover rate. In both Syrian hamsters (21) and European hamsters (12), levels of 5-hydroxyindole-3-acetic acid (5-HIAA), the primary metabolite of 5-HT, were higher in hibernating than in nonhibernating animals. In Syrian hamsters, the 5-HT turnover rate was found to be 24 times higher during than before hibernation (21). Strong evidence for a role of 5-HT in hibernation is that animals do not enter hibernation after the anterior portion of the median raphe nucleus is lesioned, abolishing a serotonergic projection to several brain areas including the hippocampus (9). Importantly, injection of 5-HT (0.8 µg) into the preoptic and anterior area of the hypothalamus (5), but not in the midbrain reticular formation (6), initiated complete arousal in the hibernating squirrel, showing 1) an action of 5-HT in hibernating animals and 2) that the effects depend on the site of injection. Taken together, these studies indicate that 5-HT can be included with neuroactive agents, such as thyrotropin-releasing hormone (7, 28), associated with hibernation (13). However, serotonergic effects on central nervous system electrophysiology of hibernating animals have yet to be fully characterized.

Specifically, the effects of 5-HT on the hamster hippocampus, a region of the central nervous system showing marked changes in EEG activity as animals enter into or arouse from hibernation (10, 13), have received limited attention. In rats, a nonhibernating species, the overall effect of this long-duration neuromodulation was first observed by applying test shocks to Shaffer collateral/commissural fibers and monitoring the effectiveness of this fast pathway in activating the hippocampal pyramidal cell population (4). With 5-HT in the bath, the afferent volley less effectively excited pyramidal cells, which generate fewer actions potentials, and as a result a smaller population spike is recorded (3, 4).

In parallel experiments on slices from euthermic Syrian hamsters (Mesocricetus auratus), 5-HT was also found to be an effective neuromodulator throughout a range of temperatures from 35 to 15°C (15). Igelmund et al. (16) have studied serotonergic modulation in a second hibernating species, the Turkish hamster (Mesocricetus brandti). 5-HT effectiveness in modulating neural activity in hippocampal slices from cold-acclimated and hibernating Syrian hamsters has yet to be determined.

To complicate the interpretation of data from studies on the slice preparation (2, 11, 16, 17), extracellular K+ concentrations in the solutions superfusing slices have ranged from 5.0 to 2.5 mM, raising the question of whether serotonergic modulation is relatively insensitive to extracellular K+ as is generally assumed. Altering K+ has been demonstrated to affect population spike amplitude in some species (25). If different values of extracellular K+ concentration also affect serotonergic modulation, K+ may exert an even more noticeable effect on the response to 5-HT recorded at lower temperatures where homeostatic mechanisms are slowed. To compare data from different laboratories thus requires consideration of the effect of changing K+ concentration on 5-HT modulation.

Although receptors activated by 5-HT have been extensively studied in the rat, receptor subtypes have not been identified in the hamster hippocampus. The rat hippocampus contains a variety of receptor subtypes (18, 22, 23), including types 1A, 1B, 1C, 2, 3, and 4. The dominant effects of 5-HT on rat CA1 pyramidal cells appear to be mediated by 5-HT1A and 5-HT4...
receptors (2, 11, 29). When 5-HT binds to a 5-HT₁A receptor, a G protein is activated and diffuses through the plasma membrane to open K⁺ channels (1). The efflux of K⁺ through open channels then hyperpolarizes CA1 pyramidal cells by a few millivolts as long as this modulatory pathway is activated (2, 11). These studies on rats suggest that experiments on hamsters may also show that 5-HT₁A receptors mediate serotonergic modulation.

In this study, rather than stepping temperature over a range of values (15, 16), the slice was held at 20°C to characterize serotonergic modulation at a low temperature encountered by an animal entering hibernation. At this temperature, a population spike could still be evoked and the following questions addressed. 1) Are there differences in the serotonergic response between euthermic, cold-acclimated, and hibernating hamsters? 2) Do changes in extracellular K⁺ concentration alter serotonergic inhibition of population spikes? 3) Is serotonergic inhibition of CA1 neurons mimicked by the 5-HT₁A receptor agonist (+)-8-OH-dipropylaminotetralin (8-OH-DPAT)?

MATERIALS AND METHODS

Hibernating and cold-acclimated animals. Male Syrian hamsters (Mesocricetus auratus) were purchased from Simonson Laboratories and housed in a room at 23°C in a 14:10-h light-dark photoperiod for at least 2 wk. All animals were provided with Simonson white diet and water ad libitum. While in the 23°C animal room, animals were housed one to four animals per cage. Animals in both the cold-acclimated and hibernator groups were transferred to a cold room kept at 6–8°C with an 8:16-h light-dark photoperiod. Animals in the cold room were housed one animal per cage. Following a 3-wk acclimation period in the cold room, hibernation status was monitored daily: the animal’s response to sound and light stimuli (opening cold room door) was observed. If an animal did not respond, the animal was picked up to further examine the animal. An animal was considered to be hibernating if it did not show a response to this tactile stimulus. Since this stimulus triggered arousal from hibernation, brain dissection was begun within 5 min of taking a hibernating animal from the cold room, before the animal aroused from hibernation. (Complete arousal at room temperature usually takes between 40 min and 2 h.)

Hippocampal slice preparation. Procedures were similar to those described previously (15). Hamsters were decapitated, hippocampi were removed bilaterally, and 400- to 450-μm slices were cut in a plane perpendicular to the long axis of the hippocampus using a McIlwain tissue chopper. Slices were placed on Ringer-saturated filter paper in a holding chamber supplied with humidified 95% O₂-5% CO₂ gas where the slices were kept at 20°C for at least 1.5 h to allow recovery. Unless stated otherwise, the Ringer consisted of (in mmol) 124.0 NaCl, 5.0 KCl, 1.3 NaH₂PO₄, 2.0 MgSO₄, 26.0 NaHCO₃, 2.0 CaCl₂, and 10.0 dextrose. Slices were transferred to the slice chamber for recording electrical activity. The slice chamber was continuously perfused with Ringer at a flow rate of 1.5–2.0 ml/min. Tissue temperature was controlled by an external refrigerated water bath (Neslab) connected to a water jacket surrounding the chamber.

Electrophysiological recordings. Bipolar tungsten-stimulating electrodes were placed in the Schaffer collateral/commissural fiber tract. A C² IBM-compatible computer controlled delivery of a 100-μs duration constant voltage pulse at 2-s intervals. Stimulus intensity was adjusted to give a baseline population spike amplitude between 30 and 80% of the maximum amplitude to allow subsequent detection of both increases and decreases in the evoked response following addition of 5-HT to the bath. For this range of baseline amplitudes (30 to 80%), regression analysis showed no correlation between the baseline amplitude and the maximum serotonergic depression (r = 0.28).

Population spikes were recorded with glass microelectrodes (tip resistance 5–15 MΩ) filled with 3.0 M NaCl inserted in the stratum pyramidale of the CA1 region. Signals from a WPI M707 microprobe amplifier were monitored on a Tektronix 7403 oscilloscope and were amplified, filtered, and converted to digital form with an analog/digital converter for averaging, display, and disk storage.

Experimental protocol. At intervals of 3–4 min, 10 or 20 evoked responses were averaged and stored. A typical population spike (Fig. 1H) is labeled to indicate the most negative voltage of the spike (site c) as well as the preceding (site a) and the following (site b) shoulder. Population spike amplitude was calculated as the average of two potentials: the voltage between sites a and c and the voltage between sites b and c. Averages were monitored until spike amplitude varied by less than 10% over a 10-min period. The tissue was then perfused with Ringer containing a known concentration of 5-HT creatinine sulfate (Sigma) or 8-OH-DPAT (Research Biochemicals). In 8-OH-DPAT experiments, the tissue was perfused with Ringer containing the drug for 20 min, and the perfusing solution was then switched back to agonist-free Ringer.

Fig. 1. CA1 pyramidal cell response in a hippocampal slice from a hibernating animal. Each trace in top set of selected waveforms shows the average of 10 responses evoked by electrical stimulation of the stratum radiatum. The vertical and horizontal scales below F show amplitude by the 0.5-mV vertical bar (positive potentials plotted upward) and time by the 5-ms horizontal bar. Trace H: an initial downward deflection (the shock artifact) followed by a population spike labeled abc (with an amplitude equal to the average of the potential from a to c and from c to b). The amplitudes of the selected population spikes in the top set of waveforms are identified at bottom by corresponding letters on the plot of all population spike amplitudes recorded throughout the experiment. Perfusion with 10 µM serotonin (5-HT; black bar) depressed population spike amplitude (sites D–F). Tissue temperature was maintained at 20°C in Figs. 1–6.
Population spikes were collected for 30 min during the washout period. Following removal of 8-OH-DPAT from the perfusion solution, population spike amplitude generally did not fully recover, probably because of the strong binding of the drug to the receptor.

In 5-HT experiments, the tissue was perfused with Ringer containing the drug for 8–20 min. The drug was applied for a sufficient time to allow serotonergic inhibition to steadily increase and then stabilize. When the inhibitory response had stabilized or when a delayed serotonergic excitation began to mask the inhibition, the perfusion solution was switched back to 5-HT-free Ringer. Population spikes were collected during the washout period until the amplitude of the population spike had peaked and was returning to control levels.

Chamber temperature was maintained at 20 ± 1°C because population spike amplitudes steadily declined at lower temperatures, although 5-HT could still evoke a depression. For example, in one slice from a hibernating hamster with bath temperature maintained at 15°C, perfusion of 10 µM 5-HT led to an 86% decrease in population spike amplitude.

The magnitude of the response prior to drug administration was taken as the control amplitude and assigned a value of 100% for comparison with subsequent responses. The smallest population spike recorded during drug application was used to calculate the maximum inhibition during that trial (expressed as a percent of control amplitude). Enhancement of the population spike following drug removal was defined as the amplitude of the largest population spike recorded during the first 15 min of the washout period (expressed as a percent of control amplitude). Data were compared with an unpaired two-sample t-test assuming equal variances. Differences were considered statistically significant for P < 0.05.

RESULTS

5-HT causes a depression followed by a delayed rebound in spike amplitude in slices from hibernating hamsters. Figure 1 shows the response of a CA1 pyramidal cell in a hippocampal slice from a hibernating hamster as 10 µM 5-HT was added to and later withdrawn from the perfusing medium. Compared with the control taken just before perfusion with 5-HT (site C), a large depression in CA1 cell population spike amplitude (sites D–F) was seen during 5-HT application. Within 3 min of 5-HT perfusion (site D), the population spike amplitude was depressed 96%. Seven minutes after withdrawal of 5-HT, the population spike amplitude (site H) had returned to 91% of the control amplitude (site C).

A 5-HT-induced depression of at least 50% was seen in all slices from hibernating animals. In nine slices, the population spike amplitude decreased an average of 80 ± 6% (means ± SE) following perfusion with 10 µM 5-HT at 20°C. Maximal depression occurred on average of 4 ± 0.8 min after 5-HT application.

Figure 1 was selected in part to show the robust serotonergic inhibition that could be seen in a slice from a hibernating hamster. In addition, Fig. 1 displays a representative temporal response, a depression followed by a rebound in population spike amplitude. Following the withdrawal of 10 µM 5-HT, CA1 population spike amplitude often, although not always, increased to a level greater than preperfusion values (site I in Fig. 1). The peak amplitude of the overshoot (site I) was 35% greater than control (site C) and was observed 11 min after 5-HT withdrawal. Enhancement of the population spike amplitude was usually a transitory response, and the amplitude then decreased toward the control level (e.g., site J). In experiments when the spike amplitude was monitored for more than 15 min after the peak amplitude was reached, the response returned toward control levels in 7 of 10 trials. The experiment illustrated in Fig. 1 is an example of this transient enhancement.

A population spike was considered enhanced if its amplitude was at least 10% greater than the preperfusion control level at any time within 15 min after 5-HT withdrawal. Spike enhancement was seen in seven of nine experiments. The average maximum spike amplitude, seen 11 ± 3 min after withdrawal of 10 µM 5-HT, was 114 ± 6% of the control amplitude.

5-HT is also an effective neuromodulator in slices from cold-acclimated hamsters. A similar serotonergic response occurred in slices from cold-acclimated hamsters as shown in Fig. 2. In this experiment, the population spike amplitude decreased 67% 1 min after 5-HT perfusion began (site B) compared with control amplitude (site A); the maximum inhibition of spike amplitude was 92% recorded 3 min after drug application (site C); and the population spike amplitude remained low throughout 5-HT perfusion and began recovering following drug removal (sites D and E).

A 10 µM 5-HT-induced depression of 10% or more was seen in all five slices; this depression averaged 54 ± 13%. The average depression was significantly less (P = 0.047) than the 80 ± 6% average depression seen in slices from hibernating hamsters. The peak depression in cold-acclimated hamsters was seen an average of 4 ± 0.6 min after drug application.

Figure 2 also illustrates the responses of a slice from a cold-acclimated hamster after removal of 5-HT from the perfusate. Five minutes after drug removal (site F), the population spike amplitude increased to the control (site A). The population spike amplitude then decreased and leveled off (sites G and H) at −67% (site H) of control amplitude (site A). When slices from cold-acclimated hamsters were recorded, spike recovery was seen in all trials (n = 5) after drug washout. After the population spike amplitude peaked following drug...
washout, in some trials the amplitude stabilized at or near control levels, whereas in other trials spike amplitude stabilized at a level lower than control amplitude. The average maximum value of the peak during the 15 min following removal of 10 µM 5-HT was 13 ± 9% greater than control amplitude and occurred 3 ± 0.6 min after withdrawal of 5-HT.

Comparison of serotonergic responses in slices from euthermic hamsters with slices from hibernating and cold-acclimated hamsters. Figure 3 shows responses in a slice from a euthermic hamster to repeated perfusion with 5-HT. As in hibernating (Fig. 1) and cold-acclimated (Fig. 2) hamsters, slices from euthermic animals respond to 5-HT with a depression in population spike amplitude followed by a rebound after 5-HT is removed. This robust depression and small overshoot is seen both when the potassium ion concentration of the bath is 2.5 and 5.0 mM.

The characteristics of this biphasic response at 20°C and a bath potassium ion concentration of 5.0 mM are summarized for slices from hibernating, cold-acclimated, and euthermic animals in Fig. 4. In all cases, there was marked inhibition in response to 5-HT. In slices from cold-acclimated hamsters, inhibition was significantly less (P < 0.05) than observed in hibernating hamsters. There was also a rebound in slices from all three groups, the magnitude of which did not significantly differ among groups. Thus 5-HT maintains its ability to inhibit the population spike in slices from hibernating animals, where in fact, the most robust inhibition of all the groups was seen. The serotonergic depression in slices from both hibernating and euthermic animals is similar, whereas the depression in slices from cold-acclimated hamsters, although clearly evident, is less robust.

Changes in extracellular K+ concentration do not greatly alter serotonergic inhibition. Increasing the K+ concentration from 2.5 to 5.0 mM K+ increased the population spike amplitude as shown for one slice in Fig. 3 at sites E and F. For five slices, the average increase on changing potassium concentration was 9 ± 6%. When K+ concentration was reduced from 5.0 mM K+ to 2.5 mM K+, spike amplitude decreased 30 ± 14% (n = 3). Combining these two groups, spike amplitude was 18 ± 8% greater (P = 0.17) when extracellular K+ concentration was 5.0 mM compared with the amplitude when K+ concentration was 2.5 mM. In the single slice shown in Fig. 3, a small increase in the serotonergic-induced depression of spike amplitude was seen when 2.5 mM K+ Ringer was changed to 5 mM Ringer (sites B and H, respectively). However, no significant difference in the magnitude of serotonergic inhibition was observed between slices bathed with 2.5 mM K+ compared with slices bathed with 5.0 mM K+. All slices used for this comparison were from euthermic hamsters. Responses to 5-HT at these two K+ concentrations are given as the percentage change in amplitude compared with a control taken after the slice had stabilized and just before serotonergic application.

This persistence of serotonergic inhibition was a typical response and was observed regardless of whether 5-HT (10 µM) was first applied to slices in 2.5 mM K+ and then 5.0 mM K+ Ringer (n = 3) or in slices first perfused with 5.0 mM K+ and then 2.5 mM K+ Ringer (n = 5). In the first case, 5-HT led to an average inhibition of 48 ± 18% in population spike amplitude in 2.5 mM K+ Ringer and an average inhibition of 68 ± 8% in 5.0 mM K+ Ringer, whereas in the second case, inhibition averaged 64 ± 12% at 2.5 mM K+ and 59 ± 10% at 5 mM. None of these values differed significantly, indicating no statistically significant effect of altered K+ concentration on serotonergic inhibition of CA1 population spikes (P = 0.30) and no significant effect of the order in which the two K+ concentrations were applied (P = 0.27). The data were also analyzed for effects of multiple 5-HT exposures by pooling data from all the first 5-HT application trials with all the second 5-HT application trials. The first superfusion of 10 µM 5-HT inhibited spike amplitude by an average of 34 ± 16% (n = 3) with a second superfusion of 5-HT leading to an average inhibition of 37 ± 15% (n = 3).
5-HT1A receptor subtype, slices from euthermic animals
population spike amplitude was associated with the
to determine if the depression in
spike amplitude
a slice only once (15).

8-OH-DPAT administration decreases population
spike amplitude. To determine if the depression in
population spike amplitude was associated with the
5-HT1A Receptor subtype, slices from eutherian animals
were perfused with 8-OH-DPAT, a 5-HT1A agonist. As
illustrated in Fig. 5, 30 µM 8-OH-DPAT decreased the
population spike markedly. Six minutes after application
of the agonist to the slice, the population spike amplitude
(site B) was 17% smaller than the control amplitude; at 22 min
(site D) the spike amplitude was maximally depressed (92% less than the control amplitude).
A decreased spike amplitude was seen in all experi-
ments in which a concentration of 1 µM or more of
8-OH-DPAT was used (n = 12) and in one of three slices
in which 0.3 µM 8-OH-DPAT was used (Fig. 6). This
depression in population spike amplitude increased from
5 ± 4% inhibition at 0.3 µM to 91 ± 3% inhibition
at 30 µM 8-OH-DPAT. Following removal of 8-OH-
DPAT from the perfusion media, spike amplitude
showed only a slight recovery, if any, toward baseline
amplitude (Fig. 5).

DISCUSSION

Serotonergic responses in hippocampal slices from
hibernating and cold-acclimated hamsters are similar
to those in slices from euthermic hamsters. In a previous

study on the euthermic Syrian hamster (15), a 15-min
perfusion of 10 µM 5-HT first depressed, and then
enhanced, population spike amplitude at temperatures
within the range of 15–35°C. In the present study, with
temperature held at 20°C, this characteristic biphasic
response was again regularly observed in slices from
euthermic hamsters (Figs. 1–3). The average decrease
in spike amplitude in euthermic hamster slices, 63%
in this study and 68% in a previous report (15), was
similar to the 70% decrease reported in the rat (4). The
more labile overshoot, averaging 9% in this study, was
less than the 33% previously reported for hamsters
(15), a difference that may be due to the different
protocols followed. As a whole, the overshoots reported
for the hamster were greater than the 5% overshoot
seen in rats (4), apparently reflecting a species differ-
ence.

One question considered in this study was the effect
of cold acclimation on serotonergic mechanisms in the
hippocampus. During cold acclimation, as the hamster
prepares for hibernation, the biphasic serotonergic
changes in population spike amplitude were still robust
e.g., Fig. 2) and in fact were similar to those of
euthermic hamsters (Figs. 3 and 4). Thus 5-HT contin-
ues to be a robust neuromodulator as the hamster
acclimates to cold before entering hibernation.

When the Syrian hamster enters hibernation, the
hippocampal EEG shows a series of characteristic
changes before it decreases in amplitude and can no
longer be detected in deep hibernation (10, 13). The
hippocampus, part of the limbic system, may play a role in
the systematic reorganization of the central nervous
system during entrance into hibernation (10). The
observation that animals with lesions of the serotoner-
gic median raphe nucleus do not enter hibernation (9)
strongly suggests that serotonergic modulation is impor-
tant at least for the initial entrance into hibernation. In
deep hibernation, metabolic rates are greatly de-
creased, core temperature falls near ambient tempera-
ture, and although neither neocortical nor hippocampal
EEG activity is seen in Syrian hamsters, the brain stem
maintains control over respiration and heart rate (10,
In the slice preparation, the amplitude of the population spike in CA1 pyramidal cells rapidly falls off below 20°C. The observation that, in slices from euthermic hamsters, 5-HT (15, 16) and norepinephrine (16) retain their effectiveness as robust neuromodulators at all temperatures investigated (from 35°C down to 20°C) led to a second question considered in this study, namely whether serotonergic neuromodulatory mechanisms are also maintained in animals in the hibernating state.

This in fact proved to be the case. As in slices from euthermic and cold-acclimated hamsters, those from hibernators responded to 10 µM 5-HT with a biphasic response: a decrease followed by a delayed transient enhancement of pyramidal cell population spike amplitude (Fig. 1). In fact, the average depression in slices from hibernators was significantly greater than those of cold-acclimated animals (Fig. 4), suggesting that pyramidal cells become even more sensitive to 5-HT's depressive effect during hibernation than just prior to hibernation during the cold-acclimated state. The overshoot in slices from hibernators was similar to that seen in euthermic hamsters.

In a comparison of the effects of carbachol, adenosine, dopamine, norepinephrine, histamine, and 5-HT on CA1 population spikes in the Turkish hamster and rat, Igelmund et al. (16) found both clear species differences as well as different responses to each modulator at 37 and 22°C. In contrast to the Syrian hamster (15) where population spike depression is seen, in slices from euthermic Turkish hamsters at 37°C the response to 20 µM 5-HT was a consistent spike enhancement of 53% (16). When the temperature was lowered to 22°C, a smaller and delayed enhancing effect was observed that was preceded by a 3- to 15-min decrease in spike amplitude averaging 19% in slices from euthermic Turkish hamsters. In tissue from hibernating animals, Igelmund et al. (16) found that, at 37°C, 20 µM 5-HT had no effect on population spike amplitude, although 100 µM 5-HT led to an enhancement of spike amplitude. Igelmund and co-workers thus report serotonergic responses in Turkish hamsters (16) that differ in several respects to responses in this and previous (15) studies on Syrian hamsters. Turkish hamsters hibernate more readily than Syrian hamsters, and this may account for the observation that some properties of the serotonergic response for Syrian hamsters seem closer to those of the rat, a nonhibernator. However, both hibernating species show a similar shift in serotonergic response from the euthermic state to the hibernating state, namely in hibernation there is a greater depression of population spike amplitude than in the euthermic state.

During hibernation, there may be a reorganization of 5-HT receptor activity in the hippocampal CA1 region of hamsters. It is possible that changes in serotonergic activity during the days or weeks of cold acclimation in preparation for hibernation lead to altered numbers/affinity of 5-HT1A receptors (16). The fact that in all slices from hibernating Syrian hamsters a large serotonergic inhibition was seen, whereas in slices from cold-acclimated and euthermic hamsters occasionally a less robust serotonergic inhibition was observed, suggests that the hippocampus in hibernating animals remains especially sensitive to 5-HT during hibernation.

Several mechanisms could account for the enhanced 5-HT1A receptor activation seen in hibernating hamsters, including up- or downregulation effects as well as unmasking of certain populations of 5-HT1A receptors. Beck et al. (3) have described two classes of 5-HT1A receptors in hippocampal pyramidal cells based on distinct pharmacological profiles, and they may be differentially affected by preparation for hibernation.

Moderate changes in extracellular K+ concentration do not alter the 5-HT1A response. Since the earliest studies on ions and neurons (20), extracellular K+ levels have been shown to affect diverse K+ channel currents (27). In a precise and extensive study on the effects of the ionic environment on slices from the golden hamster at 15–20°C, Rausche et al. (25) have shown that K+ changes from 3 to 5 mM depress the amplitudes of population spikes. Whole cell recordings of potassium ion channels on dorsal raphe neurons, including the inwardly rectifying K+ channel, show that channel activity is modified by 5-HT (24). Andrade and Nicoll (2) proposed that the decreased membrane resistance and an associated hyperpolarization following 5-HT binding to 5-HT1A receptors in the hippocampus was due to opening of a G protein-activated K+ channel, in part because the reversal potential of the response (about −90 mV) was close to the Nernst potential for K+. The amplitudes of K+ channel currents (27), including those of K+ channels modulated by 5-HT (1, 2, 24), are all directly dependent on the extracellular K+ concentration, which contributes to the driving force on K+. To place our results in context with the studies that have used K+ concentrations ranging from 2.5 to 5.0 mM (2, 11, 14–17, 25) and to determine if there were appreciable potassium-dependent changes in population spike amplitude modulation by 5-HT, we compared responses at extracellular K+ concentrations of 2.5 and 5.0 mM.

At both 2.5 and 5.0 mM K+, 5-HT elicited a robust depression in the amplitude of CA1 pyramidal cell population spikes. The absence of a significant effect on modulation of population spike amplitude after doubling the extracellular K+ concentration may be explained by the counteracting effects of changes in K+ concentration. The resting membrane potential would shift to a more depolarized value when extracellular K+ concentration is doubled from 2.5 to 5.0 mM, and this shift closer to the threshold for triggering action potentials would lead to a higher probability of cells firing and hence larger population spikes. However, the shift in membrane potential may be at least partially compensated by additional effects, including a higher percentage of inactivated Na+ channels at the more depolarized potential that would make the cells less excitable. Thus the net effect of changing bath K+ concentration may result in a relatively small change in sensitivity of the CA1 cells to afferent inputs that is masked by the larger effects due to 5-HT binding at various receptors.
on pyramidal cells. Our finding that doubling the K⁺ concentration had no significant effects on the percent change in serotonergic depression of population spike amplitude allows us to compare data in various serotonergic slice studies despite different bath K⁺ concentrations used.

The 5-HT₁₃ receptor is responsible for population spike inhibition. One consistently observed effect of 5-HT in the rat is inhibition of population spikes in the CA1 region (3, 26). Beck et al. (3) concluded that in the rat this inhibition is mediated through the 5-HT₁₃ receptor since it is mimicked by the 5-HT₁₃ agonist 8-OH-DPAT and blocked by the 5-HT₁₃ antagonist spiperone.

Further characterization of serotonergic modulation in rat CA1 neurons demonstrated that 5-HT evokes a biphasic effect on membrane potential in CA1 pyramidal cells, an initial hyperpolarization followed by a delayed depolarization (2, 11). The hyperpolarization is accompanied by a decrease in membrane resistance while the depolarization is accompanied by an increase in resistance. In addition, the delayed depolarization inhibits the slow afterhyperpolarization seen following a burst of action potentials (2, 11).

There is a general consensus that the 5-HT-induced hyperpolarization in rat CA1 pyramidal cells is mediated by the 5-HT₁₃ receptor. The hyperpolarization is blocked by the 5-HT₁₃ antagonist spiperone (2, 11). The 5-HT₁₃ agonist 8-OH-DPAT hyperpolarizes CA1 cells and decreases their membrane resistance. One effect of serotonergic binding to 5-HT₁₃ receptors is to activate a pertussis toxin-sensitive G protein that diffuses in the membrane to open a K⁺ channel (1). There is, however, no evidence linking inhibition of adenylyl cyclase to the 5-HT₁₃ receptor activation of a K⁺ conductance.

In this study, the 5-HT₁₃ receptor agonist 8-OH-DPAT was effective in inhibiting population spike amplitude in hamster CA1 neurons, an observation consistent with the assertion that the serotonergic inhibition is mediated through the 5-HT₁₃ receptor in the hamster. However, 8-OH-DPAT binding to 5-HT₁₃ receptors is less reversible than 5-HT binding, as demonstrated by the minimal recovery in spike amplitude observed following 8-OH-DPAT washout (Fig. 5). As shown in Fig. 6, population spikes were inhibited by 8-OH-DPAT over the range of concentrations from 0.3 to 30 µM with nearly complete inhibition of the population spikes by 30 µM 8-OH-DPAT (Fig. 5). These results demonstrate that the inhibition of hamster CA1 pyramidal cell excitability by 5-HT is mediated by the 5-HT₁₃ receptor.

This study was supported by National Aeronautics and Space Administration Grant NAG2–788. Address for reprints: J. M. Horowtiz, Sect. of Neurobiology, Physiology and Behavior, Univ. of California, Davis, CA 95616. Received 6 August 1996; accepted in final form 19 May 1997.

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