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Corticosteroid Modulation of Hippocampal Potentials: Increased Effect with Aging

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Adrenal steroids bind specifically to hippocampal neurons under normal conditions and may contribute to hippocampal cell loss during aging, but little is known about the neurophysiological mechanisms by which they may change hippocampal cell function. In the present studies, adrenal steroids have been shown to modulate a well-defined membrane conductance in hippocampal pyramidal cells. The calcium-dependent slow afterhyperpolarization is reduced in hippocampal slices from adrenalectomized rats, and it is increased after in vivo or in vitro administration of the adrenal steroid, corticosterone. Calcium action potentials are also reduced in adrenalectomized animals, indicating that the primary effect of corticosteroids may be on calcium conductance. The afterhyperpolarization component reduced by adrenalectomy is greater in aged rats than in young rats, suggesting that, with aging, there is an increased effect of corticosteroids on some calcium-mediated brain processes. Because elevated concentrations of intracellular calcium can be cytotoxic, these observations may increase the understanding of glucocorticoid involvement in brain aging as well as of the normal functions of these steroids in the brain.

The finding that some brain regions, particularly the hippocampus and related structures, are rich in specific corticosteroid receptors (*1*) has led to considerable speculation and research on the possible functions of corticosteroids in the brain (*2*). Hippocampal corticosteroid receptors are important for negative feedback regulation of adrenocorticotropic hormone (ACTH), for behavioral functions, and for the synthesis of several neuronal or glial proteins (*2*). Some electrophysiological studies have found that corticosteroids reduce ongoing electrical activity (*3*), whereas others have found apparent strengthening of synaptic function (*4*). In addition, rapid membrane effects (generally hyperpolarization) of steroids on hypothalamic or other neurons (*5*) have been described. However, the specific neurophysiological mechanisms through which corticosteroids modify neuronal function are still not well understood.

Corticosteroids also have been implicated in hippocampal aging. That is, under stressful conditions, corticosterone concentrations are elevated in aging rats (*6*), and long-term corticosteroid exposure may contribute to aspects of hippocampal aging, including neuronal loss and astrocyte reactivity (*7*). In addition, neuronal calcium homeostasis appears to be altered with aging. Calcium-dependent afterhyperpolarizations (AHPs) and Ca^{2+} action potentials are prolonged in aged rat hippocampal neurons, probably because of changes in membrane Ca^{2+} conductance (G_{Ca}) (*8*). There is also evidence that other aspects of Ca^{2+} homeostasis may be disturbed in the aging nervous system (*9*), including evidence of reduced Ca^{2+} clearance from terminals (*10*). Moreover, nimodipine, a Ca^{2+} channel antagonist, counteracts age-related decreases in a number of behavioral and plasticity functions (*11*).

In the present studies, we tested the hypothesis that corticosteroids modulate neuronal G_{Ca} (*12*); we measured the effects of adrenalectomy (ADX) and corticosterone (CORT) on the activation of the slow AHP,

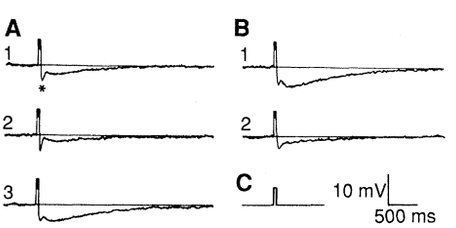

Fig. 1. Representative intracellular recordings showing current-induced bursts of three Na^+ action potentials (cut off at the top for illustration purposes) and subsequent AHPs in CA1 pyramidal cells of hippocampal slices. Traces are the averages of five waveforms for each cell shown. (**A_1**) Young, intact rat cell; (**B_1**) aged, intact rat cell; (**A_2**) young, ADX rat cell; (**B_2**) aged, ADX rat cell; and (A_3) cell from a young, ADX rat that had received CORT replacement injections. (**C**) Current trace showing example of intracellular depolarizing constant current pulse (40 ms, 0.15 to 0.25 nA) used to elicit three action potential bursts in all cells. Asterisk indicates peak of mAHP.

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Table 1. Baseline membrane and synaptic properties. Means ± SEM for baseline synaptic and membrane variables from seven young, intact controls (YI); eight young, ADX animals (YX); five young, ADX animals given CORT replacement (YX + CT); five aged, intact controls (AI); and ten aged, ADX animals (AX). No significant differences were seen for any variable.

Animals	Resting membrane potential (mV)	Action potential amplitude (mV)	Input resistance (megohm)	EPSP amplitude at action potential threshold* (mV)	Holding potential during AHP measurement (mV)	Threshold membrane deflection† (mV)
YI	−60.2 ± 1.1	88.4 ± 2.3	37.6 ± 2.6	14.9 ± 0.9	−54.2 ± 1.1	5.7 ± 0.3
YX	−60.6 ± 1.4	91.8 ± 2.1	38.4 ± 2.9	13.8 ± 0.6	−54.7 ± 0.2	5.0 ± 0.2
YX + CT	−59.4 ± 0.6	90.9 ± 2.2	40.6 ± 2.7	13.9 ± 0.8	−56.0 ± 0.6	5.4 ± 0.2
AI	−60.2 ± 0.5	92.3 ± 1.2	43.8 ± 3.1	15.4 ± 1.3	−54.6 ± 0.8	6.4 ± 0.2
AX	−60.7 ± 0.7	89.1 ± 2.0	40.8 ± 3.0	15.4 ± 0.8	−54.9 ± 0.4	5.9 ± 0.4

*Holding potential, −70 mV. †To elicit three action potentials.

Table 2. Effects of adrenalectomy and CORT replacement on AHP amplitude and duration. Means ± SEM of slow AHP amplitude at 600 ms after the third action potential, overall duration of the AHP, measured from the third action potential, and half-decay times (*21*); peak amplitudes of the mAHP in the same cells. Values from all cells per animal were averaged to yield one value per animal, and the number of animals per group was as follows: YI, seven; AI, five; YX, eight; AX, ten; and YX + CT, five. Overall main effects of treatments were assessed with ANOVA, and individual group contrasts were examined with Bonferroni post-hoc tests; NS, not significant.

Group	Mean ± SEM	Significant contrast	*P*
	Slow AHP		
Amplitude (mV)			
YI	2.38 ± 0.29		
AI	4.07 ± 0.26	AI > YI	<0.005
YX	1.45 ± 0.17	AI > YX	<0.001
AX	2.09 ± 0.27	AI > AX	<0.001
YX + CT	2.42 ± 0.43	AI > YX + CT	<0.01
Duration (ms)			
YI	1570 ± 164	AI > YI	<0.001
AI	2542 ± 155	AI > YX	<0.001
YX	1242 ± 49	AI > AX	<0.001
AX	1504 ± 91	YX + CT > YI	<0.001
YX + CT	2338 ± 102	YX + CT > YX	<0.001
		YX + CT > AX	<0.001
Half-decay (ms)			
YI	1016 ± 48	AI > YX	<0.001
AI	1162 ± 41	AI > AX	<0.003
YX	829 ± 23	YX + CT > YI	<0.015
AX	908 ± 31	YX + CT > YX	<0.001
YX + CT	1248 ± 78	YX + CT > AX	<0.001
	Medium AHP		
Peak amplitude (mV)			
YI	3.24 ± 0.48		NS for all contrasts
AI	4.02 ± 0.22		
YX	3.63 ± 0.43		
AX	3.82 ± 0.54		
YX + CT	4.00 ± 0.35		

a Ca^{2+}-dependent K^+ conductance, and on isolated Ca^{2+} action potentials. The AHP is directly dependent on intracellular Ca^{2+} concentrations (*13*), and the Ca^{2+} action potential reflects a relatively pure Ca^{2+} influx (*14, 15*). Because glucocorticoids are thought to work in brain by acting on the genome (*2*), and because CORT receptors appear to deteriorate in slices (*16*), in most of our experiments we manipulated corticosteroids in vivo, by ADX and injection, and subsequently prepared brain slices for electrophysiological study in vitro. However, in one group of experiments, we also applied CORT directly to hippocampal slices. Aspects of these studies have been described (*17*).

Hippocampal slices were taken from specific pathogen-free male Fischer 344 rats (from the National Institute on Aging) and were prepared and maintained with standard procedures (*18*). Our general methods for AHP and Ca^{2+} action potential analyses have been described (*8, 19*). Young adult rats in this study were 4 to 7 months old and aged rats were 24 to 27 months old. In the first analyses of the AHP we used seven young, intact controls (YI); five aged, intact controls (AI); eight young, ADX animals (YX); ten aged, ADX animals (AX); and five young, ADX animals given CORT (YX + CT). This latter group was administered two injections of corticosterone (7.5 mg per kilogram of body weight per injection, subcutaneously in peanut oil) at 14 and at 1.5 hours before study. Bilateral ADX was performed under full methoxyflurane anesthesia, local anesthetics, and near sterile conditions (*18*). Animals in the YX and AX groups were studied from 2 to 11 days after ADX; however, no clear effects of the interval after ADX on AHP were noted, and animals in the same age group with different delays after ADX were grouped together. All animals in the YX + CT group were injected with CORT and studied after an interval of at least 10 days after ADX.

The AHP analyses were carried out with intracellular pipettes (4*M* potassium acetate, 70 to 90 megohms) in CA1 pyramidal layer neurons that met these criteria: stable resting potential of at least −55 mV, input resistance of at least 25 megohms, and Na^+ action potential amplitude of at least 75 mV. Input resistance was measured with a range of hyperpolarizing current pulses (0.1 to 0.3 nA, 70 ms in duration). In addition, monosynaptic responses to stimulation from a Schaffer collateral-commissural pathway electrode were examined to assess physiological inputs.

In AHP studies, baseline measures of input resistance, of excitatory postsynaptic potential (EPSP) amplitude at action potential threshold [while holding the membrane at −70 mV to reduce inhibitory postsynaptic potential (IPSP) contributions], of resting membrane potential, and of action potential amplitude were carried out initially. Each cell was then depolarized by steady current injection and held at a potential that was 2 mV more hyperpolarized than the threshold for spontaneous Na^+ action potential firing (threshold was usually −52 to −54 mV) to maximize subsequent AHP measures. The AHPs were then elicited by

Table 3. Calcium action potential values in CA1 neurons. Values (means ± SEM) from neurons held at the most depolarized of three holding potentials. Similar results were obtained at −70 and −75 mV holding potentials, although action potentials generally were of briefer durations at more hyperpolarized potentials. Measurement procedures are as in the legend to Fig. 3. There were 10 intact animals (14 neurons) and 11 ADX animals (17 neurons). *P*, probability from the *t* test of the difference; NS, not significant.

Animals	Action potential amplitude (mV)	Plateau amplitude (mV)	Action potential width (ms)	Full duration (ms)	Area under curve (mV · ms)	Input resistance (megohm)
Intact	79.9 ± 1.3	28.0 ± 1.5	28.8 ± 3.2	331 ± 10	5712 ± 157	42.0 ± 1.9
ADX	78.4 ± 1.4	22.1 ± 1.9	19.3 ± 1.2	260 ± 10	3994 ± 218	38.5 ± 2.6
P	NS	0.02	0.02	0.0001	0.0001	NS

40-ms intracellular depolarizing current pulses, set at an intensity just sufficient to elicit three Na^+ action potentials, with the third action potential occurring at or near the offset of the current pulse. The membrane voltage deflection needed to elicit three Na^+ action potentials was also measured to control for possible threshold differences.

A prominent AHP was elicited consistently (Fig. 1A). A rapid and sharp early hyperpolarizing phase (Fig. 1A, asterisk) resembles a recurrent IPSP (*8, 13*). However, this early hyperpolarization is not Ca^{2+}-dependent or synaptically mediated, and it has therefore been termed a "medium AHP" (mAHP) to distinguish it from a "fast AHP" and from the slow AHP under examination in our study, both of which are Ca^{2+}-dependent (*20*).

We measured the peak amplitude and latency of the mAHP, amplitude of the slow AHP 200 and 600 ms after the third Na^+ action potential, the duration of the slow AHP, and the duration to half-maximal amplitude of the slow AHP. AHPs were measured both on-line and again, off-line, on a digital oscillographic system (Norland) and confirmed by two investigators (*21*). Statistical analyses were carried out with analyses of variance (ANOVA) for main effects, and individual group contrasts were examined with post hoc Bonferroni tests.

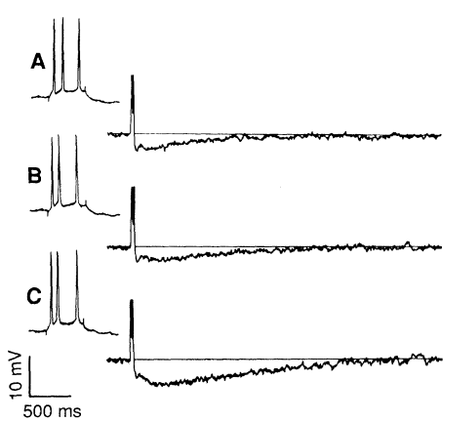

Fig. 2. Intracellular recordings from the same CA1 neuron. (**A**) Baseline period, (**B**) approximately 15 min after direct application of vehicle, and (**C**) approximately 15 min after direct application of CORT in vehicle. Each trace is a single (nonaveraged) AHP response after a three–action potential burst. The associated three–action potential burst is shown to the left on an expanded time base. Calibration marks are for traces on right. Expanded traces: 50 ms; 40 mV. CORT (2 μg) was pressure-ejected directly onto the slice in 20 μl of vehicle, giving a saturating initial dose of CORT.

The five age and treatment groups did not differ significantly across a wide range of baseline membrane and synaptic properties or in the threshold for eliciting three Na^+ action potentials (Table 1). Therefore, ADX, CORT replacement, or aging appear to exert little, if any, effect on most aspects of hippocampal cellular physiology.

However, the slow AHP values exhibited clear-cut effects of both ADX and age (Fig. 1 and Table 2): ADX reduced and aging increased AHP duration and amplitude. ADX also reduced half-decay time, although the opposing effect of aging on this variable was only of borderline significance. The mAHP was not affected either by age or ADX condition, indicating the specificity of the slow AHP effect (Table 2). Our studies replicate and extend our previous finding of an aging effect on AHP duration (*8*) by showing that aging increases AHP amplitude as well as duration.

The effect of ADX on the AHP was dependent on the removal of corticosteroids, and not on nonsteroid effects of ADX, because the YX + CT group exhibited significantly longer slow AHP duration and half-decay values than the YX and YI groups. In general, it appears that amplitude, half-decay time, and duration of the AHP tend to vary together, although there are exceptions.

With aging, the intact condition (AI) seems to reflect considerably greater activation of the CORT-dependent component of the AHP. That is, the component of the AHP that was reduced by ADX was considerably greater in neurons from aged than from young animals. This may reflect a greater sensitivity of CORT-dependent brain processes to CORT in aged animals, a greater average amount of circulating CORT in aged animals (over periods of days or weeks) (*6*), or an aging-related increase in sensitivity of the relevant membrane conductances to any form of activation (*8*).

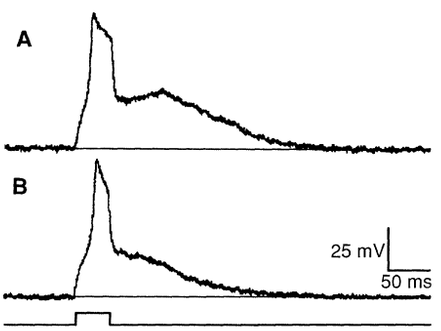

Fig. 3. Representative single Ca^{2+} action potential from Cs^+-loaded, TTX-treated CA1 neurons of young adult rats. (**A**) Young, intact cell and (**B**) young, ADX cell. Action potentials were elicited by a 40-ms depolarizing constant current pulse through the intracellular pipette. In all cases, current intensity was set at 150% of the threshold amount of current required to trigger a Ca^{2+} action potential. (**Lower trace**) Current pulse (0.5 nA) used to trigger action potential in (B). Fast action potential amplitude, fast action potential width at the base, peak plateau amplitude, overall duration from onset to return to baseline, and area under the action potential curve were quantified for cells at three holding potentials, from −65 to −75 mV.

Further support for the conclusion that the ADX effect on the AHP is mediated by CORT was obtained by direct application of steroid to hippocampal slices in vitro. After a 30-min baseline period, we pressure-ejected saturating amounts of CORT directly onto the slice (2 μg of CORT, in 20 μl of 1.0% ethanol in medium) or applied vehicle alone. The AHP was measured just before and at 10, 20, and 30 min after each application (unless the cell was lost). Saturating doses of CORT significantly increased AHP duration 30 min later in eight CORT-treated neurons when compared to eight vehicle-treated neurons (CORT: 148 ± 11% of baseline; vehicle: 109 ± 4%; $P < 0.03$, by ANOVA). Moreover, in five neurons that were stable long enough to allow measurement during a 30-min baseline period, during a 30-min period after vehicle application, and during a 30-min period after CORT application in the same neurons, the AHP duration at the 30-min CORT period was an average of 139 ± 15% of baseline, whereas AHP duration was only 108 ± 5% of baseline 30 min after initial vehicle application ($P < 0.05$, paired *t* test) (Fig. 2).

In some instances, the AHP began to lengthen as early as 15 to 20 min after application of a saturating CORT dosage (Fig. 2), but the effect for the group overall was not significant until the 30-min point. However, even 30 min is a short latency for a classical genomic-mediated steroid effect (*2*), which raises the possibility that the action of CORT on the AHP may be, at least in part, cytoplasmic or membrane mediated [although, conversely, a 15- to 30-min latency appears too long for a direct membrane mechanism (*5*)].

The slow AHP is important in the inhibitory control of neuronal excitation (*13*); therefore, this CORT-dependent component of the AHP could account for the reported inhibitory actions of corticosteroids on hippocampal and other brain activity (*3*) and might be relevant to the behavioral or ACTH negative feedback actions of corticosteroids in the hippocampus (*2*).

To test whether the changes in the AHP were due to changes in Ca^{2+} influx, we measured Ca^{2+} action potential duration and amplitude. Relatively pure, isolated Ca^{2+} action potentials can be elicited readily in Cs^+-loaded, tetrodotoxin (TTX)-treated hippocampal neurons (*14, 15, 19*), in which most confounding K^+ and Na^+ currents are blocked. In Cs^+-loaded hippocampal cells (*19*), these Ca^{2+} action potentials resemble the Ca^{2+} action potentials seen in inferior olivary neurons (*15*) (fast action potential phase followed by a longer slow phase). Calcium action potentials were measured intracellularly in CA1 neurons of slices from YI or YX rats, with CsCl-filled micropipettes (2*M*) (*14, 19*). (Cs^+ blocks the AHP.) Once the AHP was reduced substantially by Cs^+-loading, TTX was applied to the slice by pressure ejection (3 μl of 10^{-4}*M* TTX in medium). The effectiveness of the TTX application was confirmed by complete blockade of all Na^+ action potentials and of the synaptically elicited EPSP. Because Ca^{2+} action potential duration, in particular, is highly dependent on membrane potential, we measured the amplitude of Ca^{2+} action potentials at three membrane potentials in each cell, ranging from −65 to −75 mV, and confirmed the membrane potential by monitoring the potential after withdrawal from the cell. Data were collected on computer-based systems and measured off-line (Asyst package). Cells included in the Ca^{2+} action potential analyses (CsCl pipettes) had to meet the criteria for AHP cells and, in addition, had to exhibit substantial reduction of the AHP and the development of a full Ca^{2+} action potential (fast action potential and slow plateau phases).

ADX reduced Ca^{2+} fast action potential width, plateau amplitude, overall action potential duration, and area under the Ca^{2+} potential "curve" in CA1 neurons from YI animals (Fig. 3 and Table 3). In addition, in the Ca^{2+} action potential studies, a minor negative linear relation was found between days of ADX (range, 2 to 26 days before study) and some measures of Ca^{2+} action potential size (for example, plateau amplitude, slope ± SEM = 0.73 ± 0.21, $P < 0.01$; overall duration, slope = 2.4 ± 1.5, not significant).

In summary, our data indicate that corticosteroids modulate both a defined Ca^{2+}-dependent K^+ conductance and Ca^{2+} action potentials, probably by acting on voltage-dependent Ca^{2+} conductance. In addition, our results show that the impact of corticosteroids on at least one brain Ca^{2+}-dependent process appears to increase substantially with aging (whether this finding is a result of greater steroid sensitivity, greater steroid levels, or generally increased sensitivity is unclear). Therefore, our results might help explain how corticoids normally modify neuronal activity and might link evidence implicating corticosteroids in brain aging (*6, 7*) with evidence implicating Ca^{2+} homeostasis in brain aging (*8–11*). Because of the cytotoxic effects of elevated intracellular Ca^{2+} concentrations (*22*), altered Ca^{2+} homeostasis has been viewed as a potential mechanism of brain aging and Alzheimer's disease (*8, 9*).

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21. For each measurement point, five AHPs were measured on-line with a storage oscilloscope, and an average value was obtained. The same AHPs were stored on FM tape and analyzed subsequently off-line with a digital storage oscilloscope system and an averaging program (Norland). Two investigators agreed on settings for the cursors. AHP amplitude was measured at 200 and 600 ms after the third Na^+

action potential; only the 600-ms data are reported here because the 200-ms data showed similar results and were redundant. Duration measures were obtained by setting a straight line across the oscilloscope screen along the top of the baseline noise. AHP duration was calculated from the third Na^+ action potential to the point at which the top of the AHP tracing returned asymptotically to the line set along the baseline. Estimates of this point rarely varied more than 20 ms between two investigators, or between on-line and off-line values. AHP half-decay times were calculated from the maximal amplitude of the slow AHP to the point at which the amplitude had decayed to half this maximal value.

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Postponement of Satiety by Blockade of Brain Cholecystokinin (CCK-B) Receptors

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Exogenous cholecystokinin (CCK) decreases food intake and causes satiety in animals and man. However, it has not been established that endogenous CCK causes satiety or whether the response is mediated by peripheral-type (CCK-A) or brain-type (CCK-B) receptors. The development of potent and selective antagonists for CCK-A (MK-329) and CCK-B (L-365,260) receptors now allows these issues to be addressed. The CCK-A antagonist MK-329 and the CCK-B antagonist L-365,260 increased food intake in partially satiated rats and postponed the onset of satiety; however, L-365,260 was 100 times more potent than MK-329 in increasing feeding and preventing satiety. These results suggest that endogenous CCK causes satiety by an agonist action on CCK-B receptors in the brain.

THE NEUROPEPTIDE CCK DECREASES food intake in a large number of animal species and in lean and obese humans (*1*). Intraperitoneal injection of CCK causes a specific decrease in the meal size of food-deprived rats but has no effect on the water intake of water-deprived rats (*2*). Furthermore, CCK inhibits sham-feeding in rats (*3*) and monkeys (*4*), a condition in which food-deprived animals do not satiate spontaneously. In addition, CCK elicits the sequence of behavior in rats (feeding → activity → grooming → resting) that characterizes the onset of postprandial satiety (*5*). The above effects of exogenous CCK on feeding, together with the observation that CCK is released in the gut and in the brain during a meal, have led to the hypothesis that endogenous CCK is an important mediator of postprandial satiety (*1*). However, this hypothesis is controversial (*1*), and a definitive test of the involvement of endogenous CCK in satiety has been hindered by a lack of potent, selective CCK antagonists. The identification of potent, selective CCK antagonists (*6*) has now enabled us to examine the potential role of endogenous CCK in satiety.

It is also not clear if CCK decreases feeding by a peripheral or central mechanism (*1*). It has been suggested that CCK stimulates receptors in the gut that activate the vagus nerve; this signal is then thought to be relayed via the nucleus tractus solitarius (NTS) (either directly or through the parabrachial nucleus) to the hypothalamus (*7*). Thus, injection of CCK or the related decapeptide cerulein into the lateral ventricles, the ventromedial hypothalamus (VMH), and the paraventricular nucleus of the hypothalamus (PVN) has been claimed to decrease feeding, although not in all studies (*1, 8*). Furthermore, the satiety effect of CCK is blocked by vagotomy and by lesions of the NTS, VMH, and PVN (*9*). However, the blockade of CCK-induced satiety by VMH lesions has not been reproduced in all studies (*1, 9*). Ligand binding studies in rodents have shown that brain (CCK-B) receptors (which are found, for example, in the PVN, VMH, and lateral NTS) can be differentiated from peripheral (CCK-A) receptors (found, for example, in the gut, stomach, pancreas, and medial NTS) (*10*). The discovery of selective antagonists for CCK-A and CCK-B receptors (*6*) has enabled us to examine the respective roles of these receptor types in mediating CCK-induced satiety.

In the first experiment we determined the effects of access to food for periods of 0, 10, 20, 30, or 40 min on the behavioral satiety sequence in rats that had been starved overnight (*11*). As expected, increasing the length of the prefeeding period decreased food intake and accelerated the onset of the satiety sequence during the test period. A 40-min prefeeding period induced satiety almost immediately in the test period, and this condition was used to examine the potential antisatiety effects of the CCK antagonists. Doses of MK-329 [1-methyl-3-(2-indoloyl) amino-5-phenyl-3*H*-1,4-benzodiazepin-2-one] of 10 ng, 100 ng, 1 μg, 10 μg, 100 μg, or 1 mg per kilogram of body weight or an equal volume of 0.5% carboxymethylcellulose vehicle were injected subcutaneously immediately after a 40-min prefeeding period in rats that had been deprived of food for 17 hours. The animals were observed 30 min later for a period of 60 min. MK-329 significantly increased the frequency of feeding and, consequently, delayed the onset of resting (Fig. 1) (*12*). The effects of MK-329 (10 μg/kg) on the satiety sequence after a 40-min feeding period are compared to the effects of a 20- or 40-min

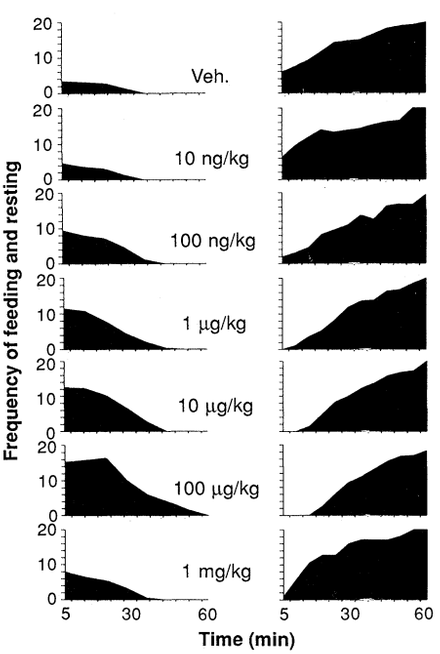

Fig. 1. Effect of MK-329 on the frequency (number of times the response is observed out of a total of 20 observations per 5-min period) of feeding (left panel) and resting (right panel) in rats deprived of food for 17 hours and then given a 40-min prefeeding period. The data are the mean of at least 12 rats per group. MK-329 significantly increased the frequency of feeding and postponed the onset of resting; two factor analysis of variance (ANOVA) with repeated measures indicated a significant main effect of MK-329 on feeding [$F(6,101) = 12.25$, $P < 0.00001$] and a significant interaction between MK-329 and time [$F(66,1111) = 5.05$, $P < 0.00001$] and a significant main effect of MK-329 on resting [$F(6,101) = 2.8$, $P < 0.02$].

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