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Haloperidol-Induced Blockade of Induction of Long-Term Potentiation in Perforant Path-Dentate Gyrus Pathway in Chronically Prepared Rabbits

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JIBIKI, I., S. WAKITA, T. KUBOTA, K. KUROKAWA, T. FUKUSHIMA AND N. YAMAGUCHI. *Haloperidol-induced blockade of induction of long-term potentiation in perforant path-dentate gyrus pathway in chronically prepared rabbits.* PHARMACOL BIOCHEM BEHAV **46**(4) 847–852, 1993.—We investigated the effects of the representative neuroleptic and dopamine receptor antagonist haloperidol (HPD) on the induction of long-term potentiation (LTP) or on the previously induced LTP in the perforant path-dentate gyrus pathway in chronically prepared rabbits. The IP HPD injection of 0.8 mg/kg blocked the induction of LTP when it was given before LTP-inducing tetanic stimulations, although this dose showed virtually no effect on the baseline control responses in the perforant path-dentate gyrus pathway to single shocks. However, neither 0.8-mg/kg nor 1.6-mg/kg HPD doses affected the previously induced LTP. The possible mechanisms underlying these results, notably the HPD-induced blockade of LTP induction, are discussed, especially in association with the inhibitory action of HPD on calmodulin-mediated events rather than dopaminergic function.

Long-term potentiation Haloperidol Calmodulin

THE long-lasting enhancement of excitatory synaptic transmission after tetanic stimulation is called long-term potentiation (LTP), which is regarded as a neuronal or synaptic plasticity. It has been generally speculated that LTP may provide a model for physiological mechanisms underlying memory and learning (1). Moreover, LTP may be related to the mechanism underlying epilepsy because LTP is similar to kindling-induced potentiation as a possible epileptogenetic underlying mechanism, although LTP is induced by weak tetanus stimulation accompanied by no afterdischarges whereas the kindling-induced potentiation is induced by more intense seizure-inducing stimulation (5,6).

It is generally known that LTP most prominently occurs in the hippocampus and, further, that LTP is induced by the activation of a glutamate receptor subtype, *N*-methyl-D-aspartate (NMDA) receptors (7). We previously reported that the activation of NMDA receptors was associated not only with the induction of kindling-induced potentiation like LTP, but also with its maintenance or expression (6), using the NMDA receptor antagonist MK 801. On the other hand, the effects of various types of neuroleptics—that is, trifluoperazine (phenothiazine neuroleptics), pimozide, spiroperidol, sulpiride, and haloperidol (HPD)—on LTP have also been studied (2,3,4,8,10). In these studies, the neuroleptics were used to examine the contribution of calmodulin-activated events and dopaminergic modulation to the induction or maintenance of LTP, since the neuroleptics are potent antagonists of the events and dopamine receptors. In particular, Krug et al. (8) studied the effects of the dopamine receptor antagonist HPD on the induction or maintenance of LTP in the perforant path-dentate gyrus of freely moving rats. However, there is a considerable conflict between their study and reports using other types of dopamine receptor antagonists, as mentioned below. We, too, are interested in the effects of HPD on LTP, since this drug is the antipsychotic most commonly used in the therapy of psychotic diseases such as schizophrenia and epileptic psychosis. So, in the present study, we investigated the effects of HPD on the induction or

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maintenance of LTP as a continuation of the previous study on the effects of the mood stabilizer and antiepileptic carbamazepine on LTP (9).

METHOD

Chronic experiments were carried out on 27 adult male rabbits weighing 2.5–3.5 kg each. Surgical procedures were conducted under IP pentobarbital sodium anesthesia (20–30 mg/kg). A tungsten microelectrode for recordings (tip diameter 1–2 μm, resistance 1–5 KΩ) and a concentric stimulating electrode for laminar analysis (0.6 mm in diameter) were attached to a holder with the tips aligned 1 mm away from each other. The tungsten microelectrode was connected to a memory oscilloscope (Nihon Kohden; VC10, bandpath 0.08–3000 Hz) through a preamplifier. After unilateral craniectomy, these electrodes were inserted from the pial surface at the position of P4 and L6 on Ridge's map to the dentate gyrus using an oil hydraulic microdrive (Narishige), with laminar analysis every 50 or 100 μm, as in the previous studies (5,6,9). The microelectrode recordings were referred to a skull screw electrode placed on the frontal sinus. In all of the 27 rabbits, the depth of the dentate gyrus measured 3500–5000 (mean ± *SD*, 4300 ± 464) μm below the pial surface. Next, another concentric stimulating electrode was inserted from the pial surface at the position of P4 and L1 to the perforant path ipsilateral to the dentate gyrus, while the maximal responses elicited in the dentate gyrus by single shocks at a constant intensity delivered from the stimulating electrode were observed. The depth of the perforant path was 3800–5300 (4730 ± 509) μm below the pial surface.

These stimulating and recording electrodes were joined together with a connector by short wires and the connector was firmly fixed to the skull with dental cement without contact with the electrodes. After a 10-day postsurgical recovery period, experiments were performed as below.

Experiment I

In 5 of the 27 rabbits, control experiments were performed to examine the magnitude and duration of LTP induced without HPD administration. The threshold intensities of single shocks to the perforant path for inducing population spikes in the dentate gyrus were initially examined. The intensities just above the threshold were determined as those of single shocks to elicit control responses which consisted of a small population spike with an amplitude of less than 0.5 mV preceded by the leading edge (population EPSP) of a slow positive wave, and the subsequent slow component (Fig. 1., Control Recording). Then, the control recording was performed for 30 min: for the initial 20 min with single stimuli at a fixed intensity (monopolar square pulses of 0.2–0.5 ms duration, 400–800 μA, 30-s stimulus interval), and for the last 10 min with single shocks at a series of four different intensities adjusted by changing the current to obtain a so-called input-output curve. Next, LTP-inducing tetanic stimulation was delivered to the perforant path. The tetanic stimulation was repeated three times at 3-min intervals because one trial of the stimulation did not necessarily induce LTP. The stimulus parameters consisted of monopolar square pulses of 0.5-ms duration, 600 μA, 60 Hz, and 1 s in total duration. Soon thereafter, single shocks at the same fixed intensity as used for the control recording were delivered again for about 2 h to observe changes in the responses in the dentate gyrus. However, for the last 10 min of the 2-h observation period, single shocks at a series of altered stimulus intensities were used only once to obtain the input-output curve.

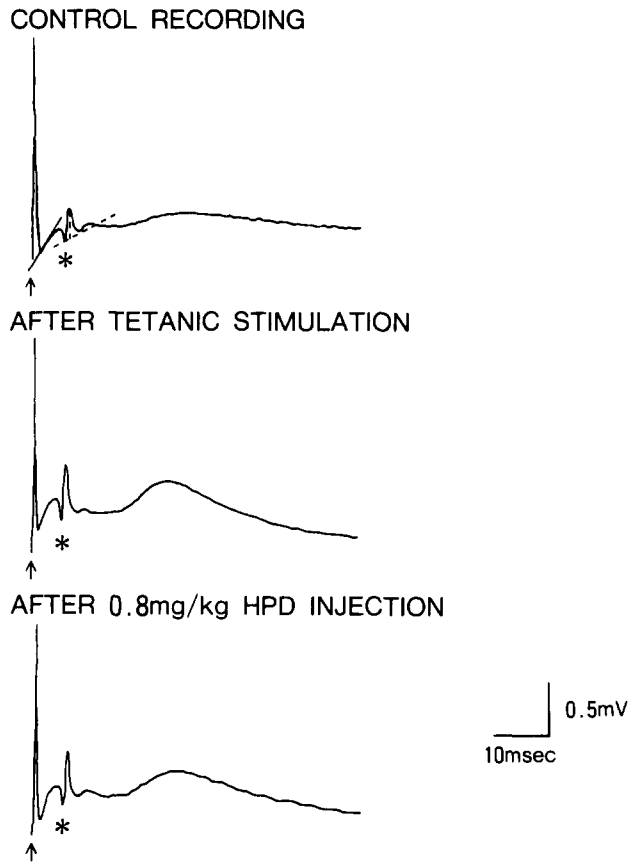

FIG. 1. A typical averaged response evoked in the dentate gyrus by single shocks at a fixed intensity to the perforant path in a single rabbit in each session in Experiment II. →: the single shock (0.2-ms pulse duration, 400 μA, 30-s stimulus interval); *: population spike. Dotted and solid lines in "Control Recording" express how to measure the population spike amplitude and population EPSP slope (slope of the leading edge of the first component in the response, mV/ms), respectively.

In 2 of the 5 rabbits, the procedure in Experiment III described below was mimicked: 1 ml of isotonic sodium chloride solution was IP injected after the control recording; at 1 h postinjection, during which period single shocks at the fixed intensity were given, the tetanic stimulation was delivered; and thereafter single shocks were given for about 2 h.

Experiment II

In 12 rabbits, experiments were performed to examine HPD effects on previously induced LTP. The control recording and subsequent tetanic stimulations were performed as in Experiment I. Then, after LTP was observed for 30 min with single shocks at the fixed and altered stimulus intensities, HPD was IP administered. High and low HPD doses of 0.8 mg/kg and 1.6 mg/kg, respectively, were used in 6 rabbits each. Soon after the HPD dose, single shocks at the fixed intensity were again delivered and the response changes were observed for about 2 h, for the last 10 min of which single shocks at the altered stimulus intensities were given. Finally,

venous blood was collected from an auricular vein to measure the serum levels of HPD.

Experiment III

In 10 rabbits, experiments were performed to examine whether LTP was induced after HPD dosing. The control recordings were initially performed as in Experiments I and II, and thereafter HPD 0.8 mg/kg was IP injected. Soon after the HPD injection, single shocks at the fixed intensity were again given to the perforant path. At 1 h postinjection, the tetanic stimulations were delivered to the perforant path as in Experiments I and II. The postinjection period of 1 h was decided in view of the literature, which describes that the maximum HPD concentration in the rat brain including the hippocampus is found at this time after the IP injection of HPD (11). Then, single shocks at the fixed intensities were again given and the responses were observed for about 2 h, also with single shocks at altered intensities given for the last 10 min. Finally, venous blood was also collected.

In each experiment, four sets of responses were averaged using a DAT1100 (Nihon Kohden) and registered with an X-Y recorder. To analyze the response changes, the amplitude of the population spike and a slope of the population EPSP were measured according to the previous studies (5,6,9), as shown in Fig. 1 (Control Recording). Further, the change in each response elicited by single shocks at the fixed intensity after the tetanic stimulation or HPD dose were expressed as a percentage of the averaged baseline amplitude or slope in all the control responses. In addition, in each rabbit after the termination of the experiment the cortical tissues at the two stimulating electrode tips were damaged by electro-coagulation, and later, these sites were identified histologically. The position of the tungsten microelectrode tip was easily determined because the microelectrode and one of the two stimulating electrodes had been placed at an identical depth, with the tips aligned.

RESULTS

Experiment I

In all 5 rabbits, the responses elicited in the dentate gyrus by single shocks at the fixed intensity to the perforant path were virtually unaltered during the control recordings before the tetanic stimulation. This was the case for the 2 rabbits injected with sodium chloride solution before the tetanic stimulation that showed no changes before or after the injection. Next, the response amplitudes were remarkably potentiated soon after the tetanic stimulations, with regard to both the population spikes and the subsequent slow potentials, although these heights fluctuated considerably. The population EPSP slope, too, increased in parallel with this potentiation. The percent changes in the population spike amplitudes and EPSP slopes in the five averaged responses—that is, 20 real responses elicited soon after the tetanic stimulations showed 131–251% (184 ± 62.8%) and 115–215% (148.3 ± 24.1%) in the total of the 5 rabbits, respectively. Such potentiated responses were consistently elicited during the subsequent observation period of 2 h. The percent changes in the population spike amplitudes and EPSP slopes in the five averaged responses elicited in the later part of the observation period showed 157–310% (218.7 ± 75.2%) and 127–208% (152.2 ± 18.3%), respectively, in the total of the 5 rabbits. However, there were no significant differences by Student's *t* test in these percent changes between the earlier and later post-tetanic periods.

Experiment II

In all 12 rabbits, the responses elicited by single shocks at the fixed intensity were also remarkably potentiated soon after the tetanic stimulations as compared with the responses in the control recording. The percent changes in the population spike amplitudes and EPSP slopes in the five averaged responses elicited soon after the tetanic stimulations showed 144–257% (200.5 ± 45.8%) and 121–151% (136.0 ± 21.2%), respectively, in the total of the 6 rabbits with the subsequent 0.8-mg/kg HPD dose. Further, they showed 144–291% (217.5 ± 57.1%) and 121–211% (166.0 ± 32.0%), respectively, in the total of the other 6 rabbits with the subsequent 1.6-mg/kg HPD dose. Next, in both rabbit groups, the responses elicited by single shocks at the fixed intensity were almost unchanged during the observation period of 2 h after the HPD doses. In the low dose group, the percent changes in the population spike amplitudes and EPSP slope in the five averaged responses in the later part of the observation period showed 143–310% (226.5 ± 65.9%) and 134–148% (141.0 ± 9.9%), respectively. Further, in the high dose group, they showed 143–298% (220.5 ± 60.6%) and 134–189% (161.5 ± 18.9%), respectively. There were no significant differences in these values between before and after HPD doses in either of the two groups. Moreover, in both of the two groups, the percent changes in the population spike amplitudes and EPSP slopes in the later part of the observation period after HPD dose showed no significant differences as compared with those in the later post-tetanic period in Experiment I.

The specimen records in Fig. 1 demonstrate the above-

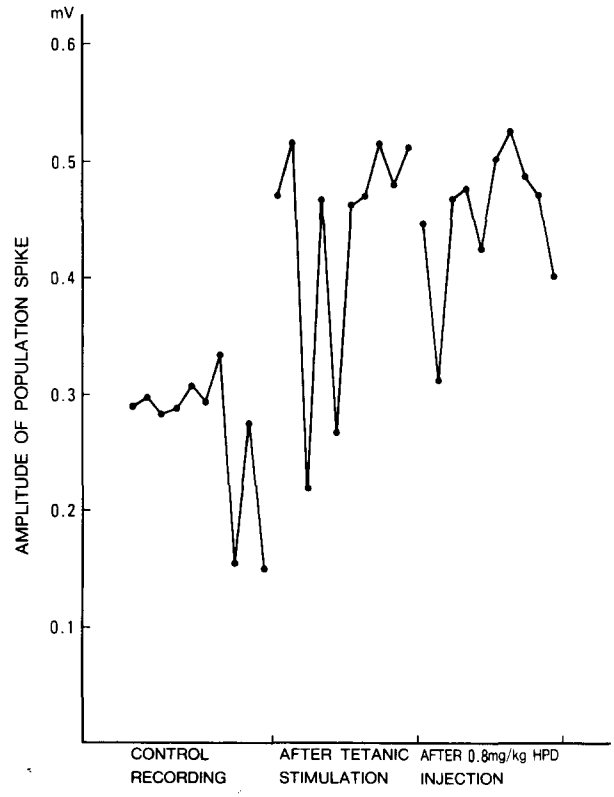

FIG. 2. Serial changes of the population spike amplitudes in the 10 averaged dentate responses elicited consecutively by single shocks at a fixed intensity in each session in the same rabbit as in Fig. 1.

mentioned response changes—in particular, the potentiated response after tetanic stimulation and unchanged response after subsequent HPD injection—observed in a rabbit of the low dose group in Experiment II. The graph in Fig. 2 shows serial changes in population spike amplitudes in the same rabbit. Further, the above-mentioned percent changes in each rabbit group in Experiments I and II are shown in Fig. 3 with

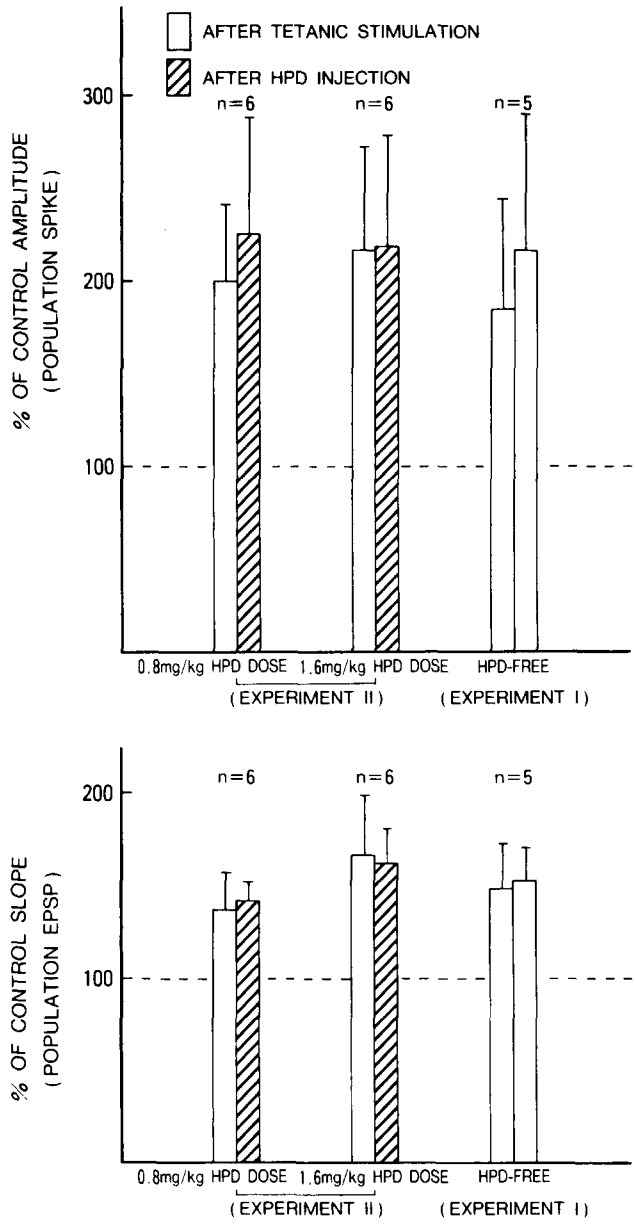

FIG. 3. Means and standard deviations of percent changes of the population spike amplitudes and EPSP slopes in the total of each rabbit groups in Experiments I and II. The percent changes in Experiment I show those in the respective five averaged responses elicited in the earlier and later periods (left and right bar graphs, respectively) after tetanic stimulations per rabbit, including data from 2 rabbits injected with NaCl solution before the tetanic stimulations. Further, the percent changes in Experiment II show those in the respective five averaged responses elicited soon after tetanic stimulations and in the later period after subsequent HPD injection per rabbit.

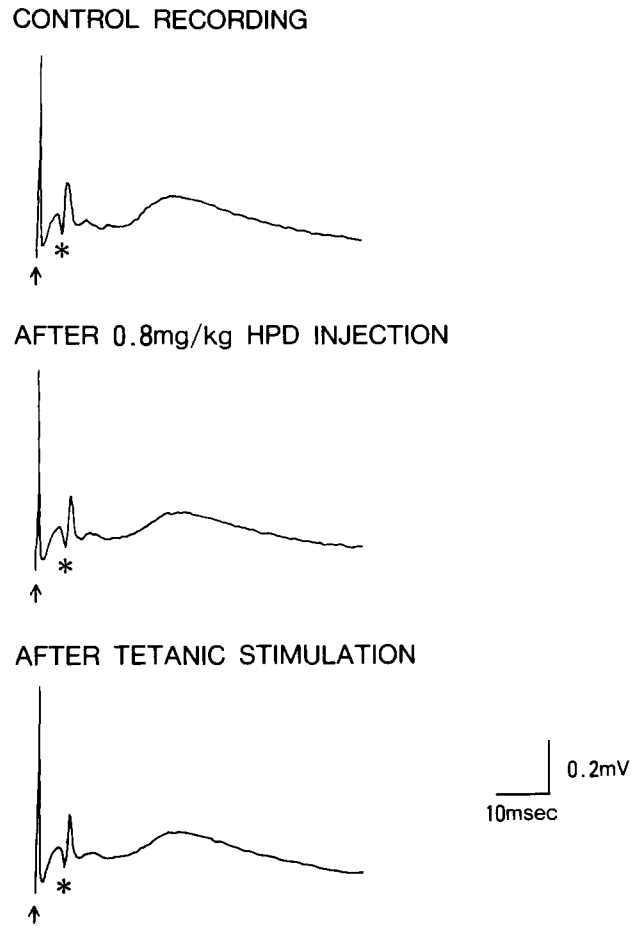

FIG. 4. A typical averaged response in each session in a single rabbit in Experiment III. →: the single shock (0.2-ms pulse duration, 400 μA, 30-s stimulus interval); *: population spike.

the means and standard deviations in the total of rabbits in each group.

In addition, in Experiment II, HPD serum levels in the venous blood collected after the termination of each experiment (i.e., at about 2 h postinjection) showed 3.2–19.2 (9.6 ± 7.2) ng/ml and 10.9–22.5 (17.5 ± 6.0) ng/ml in the rabbit groups with 0.8 and 1.6 mg/kg HPD doses, respectively.

Experiment III

In all 10 rabbits given 0.8 mg/kg HPD before the tetanic stimulations, the responses to single shocks at the fixed intensity were almost unchanged before and after the HPD dose. The percent changes in the population spike amplitudes and EPSP slopes in the five averaged responses just before tetanic stimulations showed 101–105% (102.7 ± 1.87) and 95.3–102% (98.5 ± 4.9), respectively, in the total of the 10 rabbits. Next, the responses were also virtually unchanged during the observation period of 2 h after the tetanic stimulations. The percent changes in the population spike amplitudes and EPSP slopes in the five averaged responses in the later part of the post-tetanic observation period showed 85–100% (92.3 ± 7.58) and 97.7–106% (101.5 ± 6.4), respectively. There were no significant differences in these values between before and

after the tetanic stimulations. However, these values in the post-tetanic later period showed significant differences by Student's *t*-test as compared with those in Experiment I ($p < 0.01$, $df = 13$, $t = 4.0234$ and 3.0247 regarding percent changes of the population spike amplitudes and EPSP slopes, respectively). The specimen records in Fig. 4 demonstrate such unchanged responses after HPD injection and subsequent tetanic stimulation in a rabbit in Experiment III. The graph in Fig. 5 serially plots population spike amplitudes in the same rabbit. Further, the above-mentioned percent changes in Experiment III are shown in Fig. 6 with the means and standard deviations in the total of rabbits.

In addition, in this rabbit group receiving 0.8 mg/kg HPD, the HPD serum levels at about 3 h postinjection were 2.9–5.0 (3.7 ± 1.1) ng/ml.

The input–output curves of the population spike amplitude and EPSP slopes in responses elicited by single shocks at the altered intensities showed changes consistent with the above-mentioned response changes to single shocks at the fixed intensity. Namely, when the potentiation was observed in the responses to single shocks at the fixed intensity, both the population spike amplitude and EPSP slope in responses elicited by single shocks at any of the different stimulus intensities were also potentiated as compared with those in the control responses to single shocks at each identical stimulus intensity. Further, when the responses to single shocks at the fixed intensity were almost unchanged, the input–output curves were also unaffected.

In addition, the histological examination for anatomical identification of the electrode tips showed that they had been correctly inserted into the dentate gyrus and perforant path, like in the previous study (5,6,9).

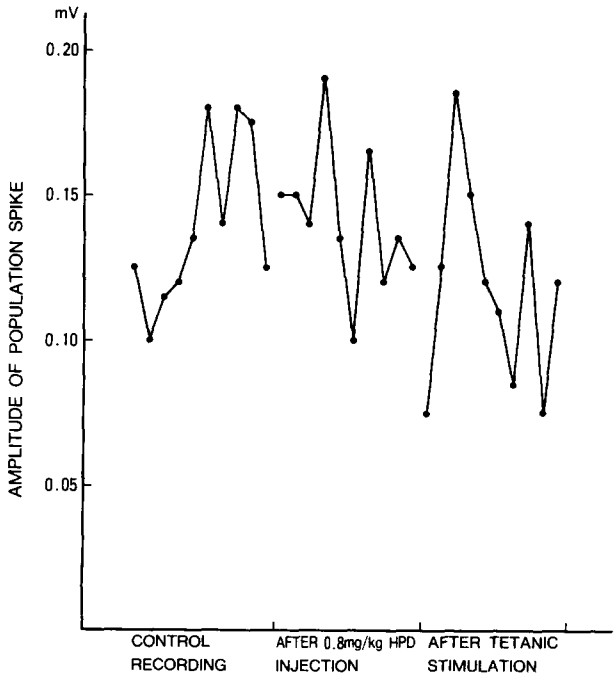

FIG. 5. Serial changes of the population spike amplitudes in the 10 averaged dentate responses elicited consecutively by single shocks at a fixed intensity in each session in the same rabbit as in Fig. 4.

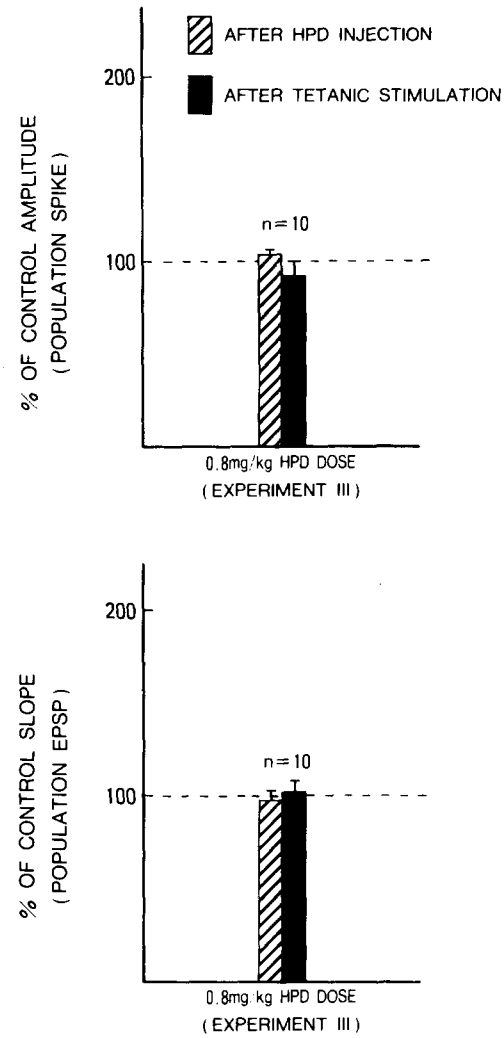

FIG. 6. Means and standard deviations of percent changes of the population spike amplitudes and EPSP slopes in the total of rabbits in Experiment III. The percent changes show those in the respective five averaged responses elicited just before tetanic stimulations following HPD injection and in the later period after the tetanic stimulations per rabbit.

DISCUSSION

The present study showed that IP HPD injection of 0.8 mg/kg blocked the induction of LTP in the perforant path-dentate gyrus pathway when it was given before LTP-inducing tetanic stimulations, although this dose had virtually no effect on the baseline control responses elicited in the pathway by single shocks. However, neither 0.8-mg/kg nor 1.6-mg/kg HPD doses affected the previously induced LTP. In a study similar to ours, Krug et al. (8) studied the effects of IP HPD injection (using, however, 0.5 mg/kg) on the baseline responses and the induction of LTP in the perforant path-dentate gyrus pathway in freely moving rats. Eventually, they found that the HPD dose showed no effects on the baseline responses like in our study, but did show facilitative effects on LTP, exhibiting more marked enhancement and prolongation of population spike potentiation when compared to that

in controls receiving NaCl solution, although the population EPSP was unaffected (8). It is known that the hippocampus receives dopaminergic afferents from the ventral tegmental area and substantia nigra (12). Therefore, they speculated that the facilitative effects might be due to the blocking of a modulatory dopaminergic influence on the granule cells in the dentate gyrus (8). Their findings on LTP completely conflict with ours. It is possible that the differences in the dosage of HPD and species of animals used in the studies account for these conflicting results.

On the other hand, in view of the previous studies on the effects of dopamine receptor antagonists other than HPD on the induction of LTP, it is known that relatively high concentrations of pimozide and trifluoperazine completely block the induction of LTP in the CA1 regions in hippocampal slices of rats (2,10). It has also been reported in a study using the same preparations that lower concentrations of the dopamine receptor antagonists domperidone and sulpiride do not block the induction of LTP but prevent the persistence or maintenance of LTP (3). It has been speculated that the former blockade of LTP induction is due to an inhibition of calmodulin activation underlying LTP induction, since the blockade did not correlate with the dopamine antagonistic potency of these drugs (2,3,10). Further, the latter prevention of LTP persistence has been attributed to the inhibition of dopamine-induced synaptic enhancement (3,4). The HPD-induced blockade of LTP induction in the present study is similar to the former findings, suggesting that the blockade results from the inhibition of calmodulin activation on account of the present higher dose of HPD. Several studies have demonstrated that various types of neuroleptic compounds are potent antagonists of calmodulin-activated enzymatic events (13). It is known that HPD, too, shows calcium-specific binding to calmodulin, although the binding is weaker than that of pimozide and trifluoperazine (13). Therefore, it is quite probable that HPD is a potent calmodulin inhibitor.

To our knowledge, there are no previous reports available on the effects of dopamine receptor antagonists on previously induced LTP. It is known from a study using hippocampal slices that dopamine has a dual effect on population responses in the CA1 region, exhibiting initially a suppression of the responses and subsequently their profound potentiation (4). Therefore, it seems unreasonable that HPD had virtually no effect on either the previously induced LTP or baseline control responses in the present study. We can offer no satisfactory explanation for these results, although the present HPD doses and experimental regions may possibly have influenced the present results.

In conclusion, the present study indicates that the higher dose of HPD blocks the induction of LTP, presumably owing to the inhibitory action of HPD on calmodulin-mediated events.

REFERENCES

1. Bliss, T. V. P.; Goddard, G. V.; Riives, M. Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. J. Physiol. 334:475–491; 1983.
2. Finn, R. C.; Browning, M.; Lynch, G. Trifluoperazine inhibits hippocampal long-term potentiation and the phosphorylation of a 40,000 dalton. Neurosci. Lett. 19:103–108; 1980.
3. Frey, U.; Schroeder, H.; Matthies, H. Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. Brain Res. 522:69–75; 1990.
4. Gribkoff, V. K.; Ashe, J. H. Modulation by dopamine of population responses and cell membrane properties of hippocampal CA1 neurons in vitro. Brain Res. 292:327–338; 1984.
5. Jibiki, I.; Fujimoto, K.; Kubota, T.; Yamaguchi, N. MK-801 (noncompetitive NMDA receptor antagonist)-induced blockade of the "burst response" in the dentate gyrus after perforant path kindling in acutely prepared rabbits. Neurosci. Lett. 116:221–226; 1990.
6. Jibiki, I.; Fujimoto, K.; Kubota, T.; Yamaguchi, N. Inhibitory effect of MK-801 (noncompetitive NMDA receptor antagonist) on kindling-induced synaptic potentiation in acutely prepared rabbits. Pharmacol. Biochem. Behav. 38:163–168; 1991.
7. Kempf, J. A.; Foster, A. C.; Wong, E. H. F. Non-competitive antagonists of excitatory amino acids receptors. Trends Neurosci. 10:294–298; 1987.
8. Krug, M.; Chepkova, A. N.; Geyer, C.; Ott, T. Aminergic blockade modulates long-term potentiation in the dentate gyrus of freely moving rats. Brain Res. Bull. 11:1–6; 1983.
9. Kubota, T.; Jibiki, I.; Fujimoto, K.; Yamaguchi, N. Facilitative effect of carbamazepine on previously induced hippocampal long-term potentiation. Pharmacol. Biochem. Behav. 42:843–847; 1992.
10. Mody, I.; Baimbridge, K. G.; Miller, J. J. Blockade of tetanic- and calcium-induced long-term potentiation in the hippocampal slice preparation by neuroleptics. Neuropharmacology 23:625–631; 1984.
11. Naylor, R. J.; Olley, J. E. The distribution of haloperidol in rat brain. Br. J. Pharmacol. 36:208–209; 1969.
12. Scatton, B.; Simon, H.; Moal, M. L.; Bischoff, S. Origin of dopaminergic innervation of the rat hippocampal formation. Neurosci. Lett. 18:125–131; 1980.
13. Weiss, B.; Wallace, T. L. Mechanisms and pharmacological implications of altering calmodulin activity. In: Cheung, W. Y., ed. Calcium and cell function, vol. 1. New York: Academic Press; 1980:329–379.