ELSEVIER

European Journal of Pharmacology 285 (1995) 79–88

ejp

Effects of the enantiomers of (±)-HA-966 on dopamine neurons: an electrophysiological study of a chiral molecule

Paul D. Shepard *, Stephen T. Connelly, Heike Lehmann, Kenneth C. Grobaski

Maryland Psychiatric Research Center and the University of Maryland School of Medicine, Baltimore, MD 21228, USA

Received 2 February 1995; revised 9 June 1995; accepted 23 June 1995

Abstract

The present study was conducted to evaluate the effects of the resolved enantiomers of (±)-1-hydroxy-3-aminopyrrolidone-2 ((±)-HA-966) on the electrophysiological properties of dopamine-containing neurons in the substantia nigra of the chloral hydrate anesthetized rat. Both (+)- and (−)-HA-966 produced a dose-dependent reduction in firing rate that eventually resulted in total cessation of spontaneous neuronal activity (ID_{50} = 5.7 and 57.8 mg/kg i.v., respectively). The inhibitory effects of both drugs were accompanied by a marked increase in the regularity of neuronal firing and a concomitant suppression of bursting activity. Although approximately 10-fold less potent than the (−) enantiomer, the inhibitory effects of (+)-HA-966 were completely antagonized by the centrally active, $GABA_B$ receptor antagonist, CGP-35348 (300 mg/kg i.v.). These data suggest that the complementary electrophysiological effects of the enantiomers of (±)-HA-966 on nigral dopamine neurons are mediated through a common mechanism of action possibly involving a novel interaction with $GABA_B$ receptors.

Keywords: Dopamine; Substantia nigra; GABA (γ-aminobutyric acid); NMDA (*N*-methyl-D-aspartate); Pyrrolidinone

1. Introduction

(±)-1-Hydroxy-3-aminopyrrolidone-2 ((±)-HA-966) was synthesized in 1959 as a chemical congener of the cyclic anhydric form of γ-aminobutyric acid (GABA) (Bonta et al., 1969b). In addition to its sedative properties, the drug is known to produce a variety of complex central nervous system actions including antitremor, muscle relaxant, anticonvulsant, antimyoclonic, anxiolytic and antinociceptive effects (Bonta et al., 1971; Menon, 1981; Corbett and Dunn, 1991). The unique behavioral profile of (±)-HA-966 prompted early speculation that the compound might be useful in the treatment of extrapyramidal disorders (Bonta et al., 1969a,b). Although the results of preliminary clinical trials proved equivocal (Van der Velden, 1969), subsequent biochemical studies have established that (±)-HA-966 is capable of markedly increasing dopamine levels in the basal ganglia without affecting forebrain levels of either norepinephrine or serotonin (Hillen et al., 1969; Bonta et al., 1971). These changes can be directly attributed to a reduction in dopamine release as well as a compensatory increase in dopamine synthesis that occurs secondary to blockade of neuronal activity within the pars compacta of the substantia nigra (Van Valkenburg and Noach, 1978; Broxterman and Mos, 1980; Shepard and Lehmann, 1992).

The complex behavioral and neurochemical profile of (±)-HA-966 can be ascribed to the disparate pharmacological effects of the individual enantiomeric forms of the drug. For example, the well documented ability of the racemic form of HA-966 to antagonize the excitatory effects of glutamate, aspartate and *N*-methyl-D-aspartate (NMDA) (Davies and Watkins, 1972, 1973; Curtis et al., 1973; Stone, 1976; Evans and Watkins, 1978; White et al., 1979) has recently been attributed to the selective actions of the (+) enantiomer (Pullan et al., 1990; Singh et al., 1990). The ability of (+)-HA-966 to attenuate the effects of endogenous excitatory amino acids is believed to reflect its activity as a low-efficacy partial agonist at the strychnine-insensitive glycine recognition site of the

* Corresponding author. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228, USA. Tel. 1.410.455.7753, fax 1.410.747.1797.

SSDI 0014-2999(95)00390-8

NMDA receptor complex (Fletcher and Lodge, 1988; Foster and Kemp, 1989; Henderson et al., 1990; Singh et al., 1990). In contrast to the seemingly well defined actions of (+)-HA-966, the identity of the receptor(s) mediating the central effects of the (−) enantiomer has yet to be identified. Similarities between the pharmacological effects of (−)-HA-966 and the prototypical $GABA_B$ receptor agonist baclofen have led to the suggestion that these receptors may be indirectly involved (Waldmeier, 1991; Shepard et al., 1993). Functionally, the (+) enantiomer is thought to be responsible for the anxiolytic and antinociceptive effects of (±)-HA-966 (Dunn et al., 1992; Anthony and Nevins, 1993; Millan and Seguin, 1993; Morrow et al., 1993; Goldstein et al., 1994) while the anticonvulsant and sedative effects are believed to be mediated principally by the (−) enantiomer (Singh et al., 1990; Vartanian and Taylor, 1991).

Both (+)- and (−)-HA-966 affect the electrical activity of mesencephalic dopamine-containing neurons, albeit to varying degrees. Thus, a single bolus dose of 30 mg/kg i.v., of the (−) enantiomer results in total cessation of spontaneous activity while an identical dose of (+)-HA-966 produces only a modest inhibition in firing rate (Shepard and Lehmann, 1992). Despite its comparatively low potency as an inhibitor of dopamine cell activity, (+)-HA-966 has other effects on these neurons including an ability to produce a marked regularization in firing pattern and a complete cessation of bursting activity (McMillen et al., 1992). Previous studies indicating that alterations in the temporal organization of dopamine neuronal spike trains can affect release from nerve terminals (Gonon, 1988; Manley et al., 1992; Suaud-Chagny et al., 1992; Nissbrandt et al., 1994) raise the possibility that some of the behavioral effects of (+)-HA-966 derive from its capacity to modify neuronal firing pattern. Such a mechanism has recently been proposed to underlie the ability of the (+) enantiomer to antagonize stress-induced activation of mesocortical dopamine neurons (Morrow et al., 1993; Goldstein et al., 1994).

Given its well documented effects as an NMDA receptor antagonist, it is tempting to speculate that the ability of (+)-HA-966 to modify dopamine cell firing pattern may arise from blockade of glutamatergic inputs to the substantia nigra. However, since the pharmacological mechanisms responsible for mediating the electrophysiological effects of (−)-HA-966 have yet to be established, we cannot exclude the possibility that both enantiomers share a common mode of action. In the present group of experiments, single unit recording techniques were used to extend earlier observations to include a comprehensive assessment of the effects of the resolved enantiomers of (±)-HA-966 on dopamine cell activity in the chloral hydrate anesthetized rat. The results indicate that electrophysiological effects previously attributed to (+)-HA-966 are shared by low, non-sedative doses of (−)-HA-966. Moreover, evidence is presented which strongly suggest that the inhibitory effects of (+)-HA-966 are mediated through an interaction with $GABA_B$ receptors. These data raise important questions regarding the specificity of action of (+)-HA-966 and suggest that some of the behavioral and neurochemical effects previously attributed to the NMDA receptor antagonist properties of the drug may actually stem from an interaction with the (−)-HA-966 binding site.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) were maintained in a constant temperature vivarium (25°C) under controlled lighting conditions (12/12 h light/dark cycle). Animals were housed in standard laboratory cages in groups of 2–3 and provided unlimited access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine and carried out in strict accordance with the principals specified in the Handbook for the Use of Animals in Neuroscience Research (Society for Neuroscience, Washington, DC, USA).

2.2. Single unit recording studies

Animals weighing 250–300 g were anesthetized with chloral hydrate (400 mg/kg i.p.) and the right femoral vein cannulated for intravenous administration of supplementary anesthetic and test drugs. Animals were positioned in a stereotaxic apparatus equipped with a feedback-controlled heating pad that maintained body temperature at 36.5°C throughout the experiment. A small incision was made in the scalp and a burr hole drilled in the skull exposing the cortex overlying the substantia nigra (2–4 mm anterior to lambdoidal suture, 1.5–3 mm lateral to midline). Incision sites and soft tissue pressure points were infiltrated with a long-acting local anesthetic (mepivacaine, 2%).

Extracellular single unit activity was recorded using epoxy-coated tungsten microelectrodes (FHC Corporation, Brunswick, ME, USA) with an impedance of 2.5–4.5 MΩ. Electrodes were stereotaxically positioned within the substantia nigra using a hydraulic microdrive. Extracellular potentials were amplified, filtered (bandpass 0.1–4 kHz) and resolved from background noise using a high impedance AC amplifier equipped with a window discriminator. Spontaneous activity of well isolated cells was monitored using both an analog oscilloscope and audio amplifier. Neurons were

prospectively identified as dopaminergic on the basis of established electrophysiological criteria (Grace and Bunney, 1980a). Single unit activity from dopamine-containing cells was observed and recorded for a minimum of 5 min to establish basal firing characteristics. Subsequently, each animal received a single dose of either (+)-HA-966 (3–100 mg/kg) or (−)-HA-966 (1–30 mg/kg) or multiple doses (300–500 mg/kg) of the centrally acting $GABA_B$ receptor antagonist, 3-aminopropane-diethoxymethylphosphinic acid (CGP-35348; Olpe et al., 1990). In some experiments, animals that had previously received a bolus injection of (+)-HA-966 (100 mg/kg) were administered a single dose of CGP-35348 (300 mg/kg). All drugs were given intravenously and only one cell was tested per animal. At the conclusion of each experiment, a microlesion was produced at the tip of the recording electrode by application of a small DC current (+20 μA for 45 s). Following removal from the stereotaxic apparatus, animals were deeply anesthetized and perfused transcardially with saline followed by neutral buffered formalin. Brains were removed, fixed and cryopreserved prior to preparing 30 μm thick frozen sections. Tissue sections were stained with cresyl violet and examined for verification of electrode placement. All cells included in the present study were localized within the pars compacta of the substantia nigra.

2.3. Data acquisition and analysis

Data acquisition and analysis was accomplished using an integrated software package for electrophysiology (RISI, Symbolic Logic, Dallas, TX, USA). Cumulative rate histograms were compiled in real-time from discriminated spikes accumulated in consecutive 10 s intervals. Analysis of neuronal firing pattern was conducted off-line from spike trains collected before and after drug administration. Unless otherwise indicated, first-order interspike interval histograms were compiled from 1000 consecutive spikes using a 1 ms bin width. Alterations in firing pattern were assessed, in part, by paired comparison of the variation coefficients associated with control and post-drug interspike interval histograms. This statistic, defined as the ratio between the standard deviation and mean interval, is relatively insensitive to alterations in firing rate and has been used successfully to quantify changes in the temporal organization of neuronal spike trains (Werner and Mountcastle, 1963; Matthews et al., 1984). Control and post-drug interval data were also analyzed using an algorithm that tallied the number of events occurring as doublets or as bursts comprised of 3 or more spikes. Bursts were operationally defined as spike pairs with an interspike interval less than or equal to 80 ms. All subsequent spikes were considered as components of the initial burst unless an interval exceeding 160 ms was encountered which signaled burst termination.

All data are presented in the text as mean ± S.E.M. Dose-response curves illustrating the inhibitory effects of the resolved enantiomers of (±)-HA-966 were compiled from groups of animals that received a single bolus dose of either (+)- or (−)-HA-966 ($n = 3$–13 cells per group). Alterations in firing rate reflecting the maximal effect of each drug were expressed as a percentage of the pre-drug (control) firing rate and plotted as a function of the dosage administered. ID_{50} values, representing the average dose required to inhibit neuronal firing by 50%, were computed using a 4-parameter logistic equation (De Lean et al., 1978). Data used to construct the dose-response relationship for (±)-HA-966 were compiled from an earlier group of experiments (Shepard and Lehmann, 1992). Omnibus testing of the overall differences between treatment groups was accomplished using a one-way analysis of variance with repeated measures or in the case of paired comparisons with a paired t-test. Post-hoc comparisons between treatment groups was conducted using the Bonferroni t-test method.

2.4. Drugs

(+)-HA-966 was obtained from Merck Sharp and Dohme Research Laboratories (Essex, UK) as a gift or purchased from Tocris Cookson (Bristol, UK). CGP-35348 was provided as a gift from Ciba-Geigy, Basel, Switzerland. (−)-HA-966 was provided by Research Biochemicals International (Natick, MA, USA) as part of the Chemical Synthesis Program of the National Institutes of Mental Health, Contract 278-90-0007 (BS). All drugs were dissolved in 0.9% saline.

3. Results

3.1. Effects of the resolved enantiomers of (±)-HA-966 on the spontaneous activity of nigral dopamine-containing neurons

All forms of HA-966 inhibited the firing rate of dopamine-containing neurons in a dose-dependent manner (Fig. 1). (−)-HA-966 proved to be the most potent of the three compounds with an estimated ID_{50} of 5.7 ± 1.2 mg/kg ($n = 37$). High doses of the drug (10 and 30 mg/kg) caused a total cessation of spontaneous activity in the majority of cells tested (9/13 and 4/5, respectively). A single dose of 6 mg/kg consistently inhibited neuronal activity but failed to silence any of the cells tested ($n = 5$). Lower doses of the drug (1 and 3 mg/kg) produced a more variable response. At the 3 mg/kg dose, 6 of 8 cells were inhibited by an average of 23.7 ± 6.0% while the remaining 2 cells showed

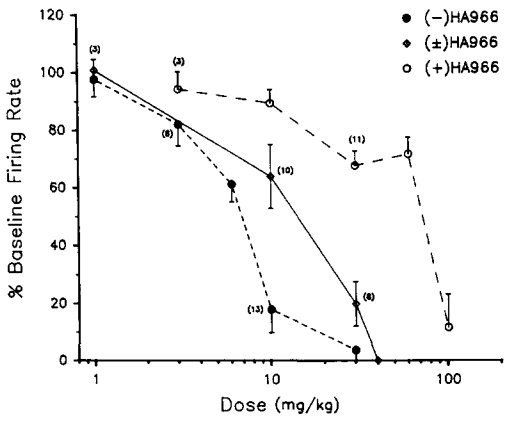

Fig. 1. Log dose-response curves illustrating the effects of (±)-HA-966 and its resolved enantiomers on the firing rate of nigral dopamine neurons. Data are expressed as the percent change in basal firing rate exhibited by groups of cells tested with a single dose of one of the 3 forms of HA-966. Each point represents the average *maximal* response (mean ± S.E.M.) of 5–6 cells unless otherwise indicated (*n*). In most cases, firing rate changes were computed 10–20 min following drug injection (see text for details regarding latency to onset). Data illustrating the effects of (±)-HA-966 were compiled from an earlier group of experiments (Shepard and Lehmann, 1992). Note that the non-sigmoidal nature of the dose-response relationship for (±)-HA-966 appears to reflect the combined profile of the individual enantiomers of the parent compound.

modest but sustained increase in firing rate (23.3 ± 8.7%). Threshold doses of the drug (1 mg/kg) increased the activity of 3 of 6 cells tested (7.9 ± 3.4%) with the remaining 3 neurons showing either a decrease in rate ($n = 1$) or no effect ($n = 2$). The average latency to onset of the peak inhibitory effects of (−)-HA-966 at 10 and 30 mg/kg averaged 11.3 ± 1.6 and 12.0 ± 5.2 min, respectively and did not differ significantly from those observed in response to lower doses of the drug (3 mg/kg: 14.7 ± 2.0 min; 6 mg/kg: 13.5 ± 1.9 min; ANOVA, $F(3,27) = 0.44$, $P > 0.7$). Furthermore, no significant relationship was observed between latency to onset and the magnitude of the inhibitory effects produced by (−)-HA-966 ($r = -0.16$, $n = 31$, $P > 0.4$).

Although the ability of (−)-HA-966 to produce a sustained increase in neuronal firing rate was confined to doses below 6 mg/kg, the inhibitory effects produced by higher doses of the drug were frequently preceded by a transient excitatory effect. This biphasic response was most frequently observed at the 10 mg/kg dose where 5 of 13 dopamine neurons displayed an initial excitation averaging 16.7 ± 2.8%.

Systemic administration of (+)-HA-966 also inhibited the firing rate of nigral dopamine neurons although higher doses were required ($ID_{50} = 57.8 \pm 1.2$ mg/kg, $n = 30$). Thus, while (+)-HA-966 administered in doses up to 10 mg/kg had no effect on neuronal firing rate (control: 2.6 ± 0.2 Hz; 3 mg/kg (+)-HA-966: 2.8 ± 0.2 Hz, $n = 3$; control: 4.9 ± 0.8 Hz; 10 mg/kg (+)-HA-966: 4.3 ± 0.6 Hz, $n = 5$), higher doses of the drug significantly inhibited the activity of all cells tested (control: 3.7 ± 0.4 Hz; 30 mg/kg (+)-HA-966: 2.6 ± 0.4 Hz, paired *t*-test, $t_{10} = 9.3$, $P < 0.0001$; control: 4.4 ± 0.6 Hz; 60 mg/kg (+)-HA-966: 3.1 ± 0.4 Hz, paired *t*-test, $t_4 = 3.1$, $P < 0.05$). Although the magnitude of the inhibition produced by 30 mg/kg of (+)-HA-966 was comparable to that observed in response to 60 mg/kg, a single injection of 100 mg/kg of the drug completely inhibited the firing rate of 5 of the 6 cells tested. Latency to onset of the maximal inhibitory effects of (+)-HA-966 (30–100 mg/kg) averaged 29.7 ± 1.9 min ($n = 22$). As a group, cells that were completely inhibited by a single dose of (+)-HA-966 exhibited a longer latency to onset of these effects than cells tested with an equivalent dose of the (−) enantiomer ((+)-HA-966, 100 mg/kg: 20.7 ± 3.6 min; (−)-HA-966, 10 mg/kg: 10.5 ± 2.2 min; Student's $t_{12} = -2.5$, $P < 0.05$). No relationship was observed between the time required to achieve maximal inhibition in activity and either the dose of (+)-HA-966 administered or magnitude of the drug-induced alteration in firing rate.

As with the (−) enantiomer, (+)-HA-966 (30 and 60 mg/kg) caused an initial transient increase in neuronal activity in approximately one-half of the cells tested (Fig. 2A). Although of comparable magnitude (19.3 ± 4.6% increase), the duration of the excitatory component was significantly longer than that observed for (−)-HA-966 (12.1 ± 1.7 min vs. 2.1 ± 0.9 min, Mann-Whitney *U*, $P < 0.003$). However, none of the cells tested with (+)-HA-966 ($n = 30$) showed evidence of a sustained increase in firing rate.

As previously reported (Shepard and Lehmann, 1992), intravenous administration of the racemic form of HA-966 inhibited the firing rate of nigral dopamine-containing neurons in a dose-dependent manner ($ID_{50} = 10.7 \pm 1.3$ mg/kg, $n = 27$). As illustrated in Fig. 1, the profile of the dose-response relationship obtained in response to (±)-HA-966 appeared to reflect the combined effects of the individual enantiomeric forms of the drug.

3.2. Effects of (+)- and (−)-HA-966 on neuronal firing pattern

Alterations in the spontaneous activity of nigral dopamine neurons following administration of the individual enantiomers of (±)-HA-966 were accompanied by a pronounced change in neuronal firing pattern. As illustrated in Fig. 2, both drugs produced a 'pacemaker-like' discharge pattern characterized by interspike interval distributions consisting of a normally distributed but markedly leptokurtic primary peak. Clusters of intervals forming smaller secondary and tertiary distributions frequently appeared at longer harmonic intervals and could be attributed to periodic

spike failure (Fig. 2B and E). Decreases in the dispersion of intervals comprising individual distributions were reflected by a significant reduction in the average variation coefficient computed for all cells that remained spontaneously active following drug administration (Fig. 3). Drug-induced increases in the regularity of neuronal firing pattern were also associated with

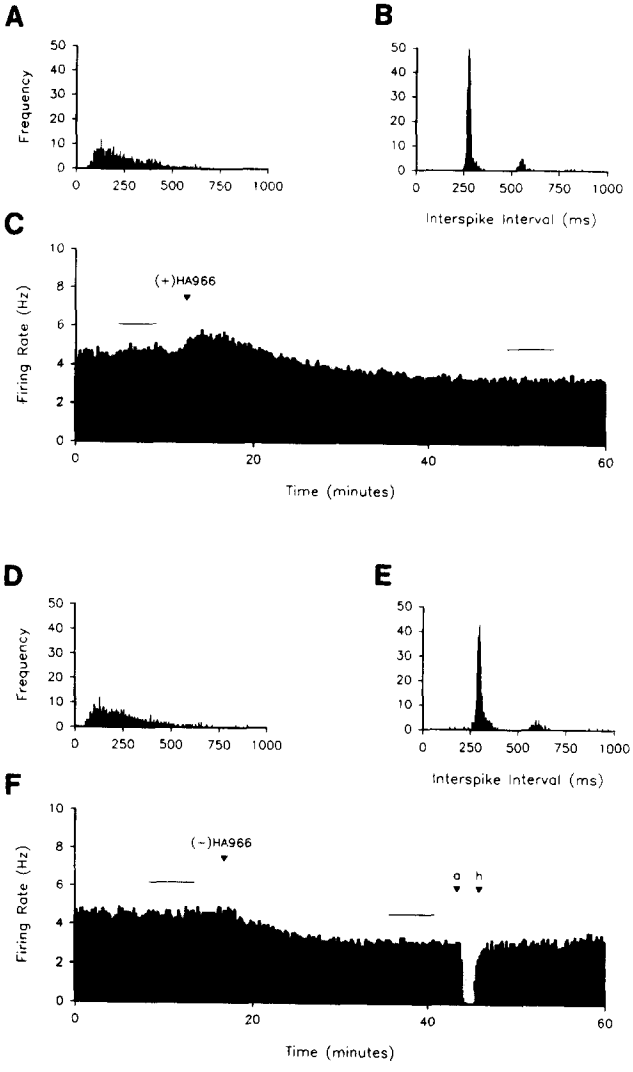

Fig. 2. Effects of (+)- and (−)-HA-966 on the firing properties of nigral dopamine-containing neurons. A–C: Integrated rate (C) and associated interspike interval histograms (A and B) illustrating the effects of a single injection of 6 mg/kg of (+)-HA-966 on the activity of a representative dopamine neuron. Note the transient increase in firing rate preceding the inhibitory effects of the drug. D–F: Effects of (−)-HA-966 (6 mg/kg) on the firing rate (F) and discharge pattern (D and E) of a typical dopamine neuron. Inverted arrowheads denote drug injection (a = apomorphine, 40 μg/kg; h = haloperidol, 0.1 mg/kg). In both examples, horizontal lines on the rate histograms denote the segments of data used to compile pre-drug (A and D) and post-drug (B and E) interval distributions ($n = 1000$ spikes, 1 ms bin width). Note that the modest inhibitory effects of both drugs were associated with a pronounced reduction in the variability of neuronal firing.

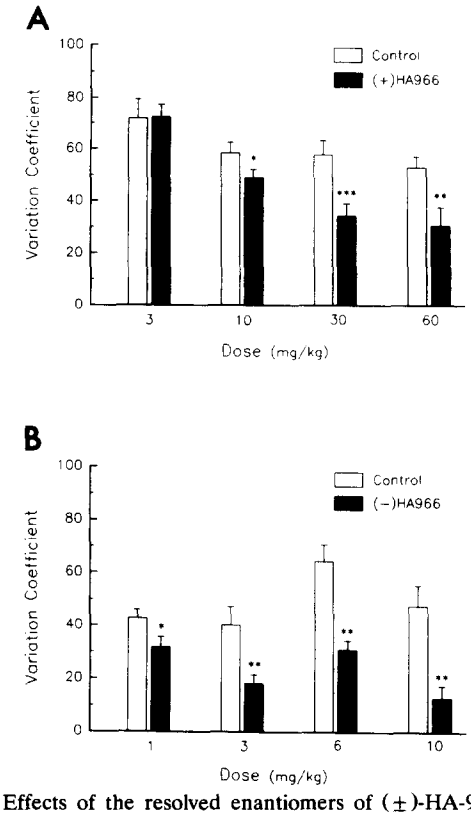

Fig. 3. Effects of the resolved enantiomers of (±)-HA-966 on the firing pattern of dopamine-containing neurons. Variation coefficients were calculated from interspike interval histograms ($n = 1000$ spikes) compiled immediately before (open bars) and within 30 min after (solid bars) a single intravenous injection of A: (+)-HA-966 or B: (−)-HA-966 at the dosages indicted. Data are expressed as mean ± S.E.M. (+)-HA-966: 3 mg/kg, $n = 3$; 10 mg/kg, $n = 5$; 30 mg/kg, $n = 11$; 60 mg/kg, $n = 5$. (−)-HA-966: 1 mg/kg, $n = 6$; 3 mg/kg, $n = 7$; 6 mg/kg, $n = 5$; 10 mg/kg, $n = 4$. Asterisks denote significant differences from control values (paired t-test). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

a dose-dependent reduction in the percentage of spikes occurring in bursts (Table 1). Notably, doses of (−)- and (+)-HA-966 previously shown to have no effect on firing rate (1 and 10 mg/kg, respectively) were found to significantly modify neuronal firing pattern. Although qualitatively similar, comparison of the magnitude of the changes produced by the individual enantiomers of (±)-HA-966 revealed the (−) enantiomer to be approximately 10-fold more potent in regularizing firing pattern than (+)-HA-966.

3.3. Effects of CGP-35348 on (+)-HA-966-induced alterations in neuronal activity

Previous studies indicating that the inhibitory effects of (−)-HA-966 can be reversed by CGP-35348 have provided evidence for the involvement of $GABA_B$ receptors in mediating the unique electrophysiological effects of the (−) enantiomer (Shepard et al., 1993).

Table 1
Effects of (+)- and (−)-HA-966 on bursting activity of nigral dopamine-containing neurons

Drug mg/kg i.v.	n	Doublets		> 3 spikes		% Decrease [a] (total bursting)
		Control	Post-drug	Control	Post-drug	
(+)-HA-966						
3	3	352	332	185	222	−3.0
10	5	370	198	844	277	60.9
30	11	804	0	443	0	100
60	5	528	0	259	0	100
(−)-HA-966						
1	6	318	52	77	6	85.0
3	7	346	6	953	0	99.5
6	5	588	0	252	0	100
10	4	316	0	144	0	100

Values represent the total number of spikes occurring as doublets or as bursts comprised of 3 or more spikes. Data were derived from interspike interval histograms comprised of 1000 consecutive action potentials obtained from cells that remained spontaneously active following drug administration. [a] Decrease in total bursting (doublets + > 3 spikes) expressed as a percentage of pre-drug control values.

Similarities observed in the present study between the effects of the resolved enantiomers of (±)-HA-966 on dopamine cell activity prompted us to test whether CGP-35348 was also effective in antagonizing the electrophysiological effects of (+)-HA-966. As illustrated in Fig. 4, systemic administration of CGP-35348 (300–500 mg/kg) alone failed to alter the basal activity of dopamine-containing neurons (control: 4.8 ± 0.3 Hz; CGP-35348, 300 mg/kg: 4.6 ± 0.2 Hz; CGP-35348, 500 mg/kg: 4.6 ± 0.3 Hz; $n = 6$, ANOVA, $P > 0.3$). Neuronal firing pattern was also unaffected by the drug (coefficient of variation, control 39.3 ± 11.4%; CGP-35348, 300 mg/kg: 48.3 ± 10.4%; CGP-35348, 500 mg/kg: 46.8 ± 7.3%; $n = 6$, ANOVA, $P > 0.1$). However, as illustrated in Fig. 5, when administered following a dose of (+)-HA-966 capable of completely inhibiting dopamine cell firing (100 mg/kg), CGP-35348 (300 mg/kg) caused a rapid return of spontaneous activity in all cells tested ($n = 5$). Average firing rates following CGP-35348-induced reversal of the inhibitory effects of (+)-HA-966 were not significantly different from control values (control: 4.6 ± 0.3 Hz; CGP-35348, 300 mg/kg: 4.4 ± 0.9, $n = 5$, Bonferroni t-test, $P > 0.05$). Despite the ability of CGP-35348 to fully antagonize the rate suppressant effects of (+)-HA-966, the firing pattern exhibited by cells following the antagonist was markedly pacemaker-like (Fig. 5B). This was reflected by a significant reduction in the coefficient of variation associated with interspike interval histograms

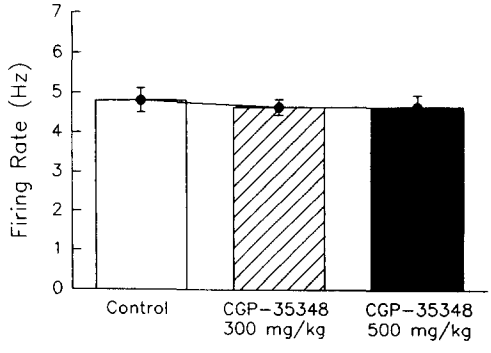

Fig. 4. Effects of CGP-35348 on the firing rate of nigral dopamine-containing neurons. Vertical bars represent the average firing rate (±S.E.M.) of 6 neurons immediately prior to and within 20 min following 2 consecutive injections of CGP-35348 (300 mg/kg + 200 mg/kg) totaling 500 mg/kg. Note that neither dose of the drug affected basal discharge rate.

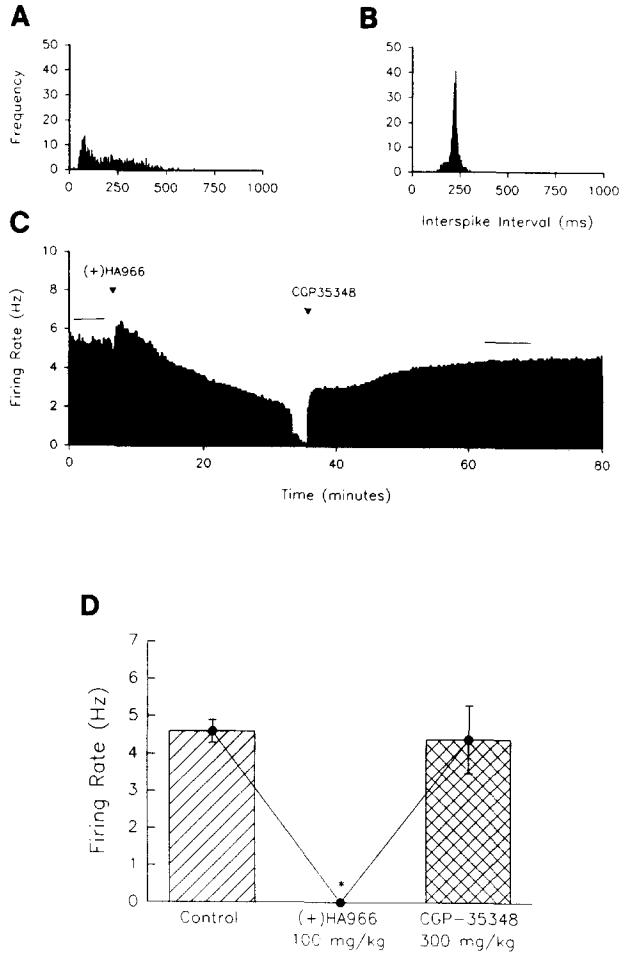

Fig. 5. Effect of CGP-35348 on (+)-HA-966-induced inhibition in dopamine cell impulse flow. A–C: Representative rate (C) and interspike interval histograms (A and B) illustrating the effects of a single intravenous injection of CGP-35348 (300 mg/kg) on the activity of a nigral dopamine cell following inhibition by (+)-HA-966 (100 mg/kg). Horizontal lines above the rate histogram denote areas used to compile the interspike interval histograms. Arrows indicate time of drug injection. Note the marked increase in regularity of firing following CGP-35348-induced recovery of neuronal activity. D: Bar graph summarizing the results from all experiments ($n = 5$). Data are expressed as the average firing rate in Hz (mean ± S.E.M.). * $P <$ 0.05, Bonferroni t-test.

($n = 500$ events) compiled following CGP-35348-induced reversal of the inhibition produced by (+)-HA-966 (coefficient of variation, control: $61.7 \pm 6.1\%$; CGP-35348, 300 mg/kg: 12.3 ± 2.3; paired $t_4 = 8.6$, $P = 0.001$).

4. Discussion

Previous electrophysiological studies have established the ability of (±)-HA-966 to inhibit the spontaneous activity of mesencephalic dopamine-containing neurons in vivo (Shepard and Lehmann, 1992). When administered in sufficiently high doses, the drug is capable of temporarily blocking neuronal impulse flow leading to a compensatory elevation in dopamine levels in the forebrain (Bonta et al., 1971; Hillen and Noach, 1971). Neurochemical studies comparing the effects of the resolved enantiomers of (±)-HA-966 on striatal dopamine levels have attributed these effects to the actions of (−)-HA-966 (Singh et al., 1990). In agreement with these findings, we have previously reported that the ability of (±)-HA-966 to reversibly suppress dopamine cell activity resides principally with the (−) enantiomer (Shepard and Lehmann, 1992). However, since both of the aforementioned studies were limited to comparison of a single dose of the resolved enantiomers of (±)-HA-966, the relative potency and efficacy of these drugs could not be established with certainty. The results of the present study, while confirming the superior potency of (−)-HA-966 in inhibiting neuronal activity, have revealed that both (+)- and (−)-HA-966 are capable of blocking dopamine cell impulse flow. Overall, (+)-HA-966 was found to be approximately 10-fold less potent than (−)-HA966 (ID_{50}: 57.8 vs. 5.7 mg/kg, respectively). Nevertheless, a single dose of 100 mg/kg of the (+) enantiomer was sufficient to completely suppress the activity of the majority of cells tested. Comparable effects were observed following 10 mg/kg of (−)-HA-966. Inhibitory effects similar to those described in the present study have not been observed in response to either local or systemic administration of NMDA receptor antagonists (Tung et al., 1991; Overton and Clark, 1992; Chergui et al., 1993; French et al., 1993; Shepard et al., 1994). To the contrary, some of these drugs have been reported to increase dopamine cell activity (French and Ceci, 1990; French et al., 1993). Taken together, these data strongly suggest that mechanisms other than blockade of NMDA receptors are involved in mediating the rate-suppressant effects of (+)-HA-966.

Although (+)- and (−)-HA-966 proved capable of markedly inhibiting dopamine cell activity, the effects of both drugs required 10–20 min to become fully expressed and were occasionally preceded by a transient excitation. The latter observation together with the finding that some cells tested with low doses of (−)-HA-966 exhibited a sustained elevation in firing rate suggest that the inhibitory effects of (+)- and (−)-HA-966 may mask an underlying excitatory effect. In fact, γ-hydroxybutyrate, a drug with many of the same neurochemical and behavioral actions as (−)-HA-966 including the ability to suppress dopamine cell activity in the chloral hydrate anesthetized rat (Walters et al., 1972; Roth et al., 1980; Engberg and Nissbrandt, 1993) has been shown to excite dopamine cells in an unanesthetized preparation (Diana et al., 1991). Moreover, preliminary studies conducted in our laboratory have revealed that the magnitude of the inhibitory effects of (−)-HA-966 are significantly reduced in ketamine-anesthetized animals (Shepard et al., 1993). Thus, it is conceivable that the rate-decreasing effects of the resolved enantiomers of (±)-HA-966 involve a synergistic interaction with chloral hydrate.

The pharmacological basis of the relatively long delay in onset of the inhibitory effects of the resolved enantiomers of (±)-HA-966 is at present unknown but may provide important insights into the mechanisms responsible for producing the unique electrophysiological effects of these drugs. In addition to potential pharmacokinetic considerations (bioavailability, first pass effect, etc.), it is possible that the enantiomers of (±)-HA-966 like their chemical congener, γ-butyrolactone, are converted to an active form by enzymatic degradation in vivo (Roth and Giarman, 1966; Gessa et al., 1966). Such a mechanism has been proposed to explain the delay in onset of the behavioral effects of (±)-HA-966 (Bonta et al., 1971). Optical specificity of the catalytic enzyme for its substrate may favor conversion of the (−) enantiomer and thus explain both the delay in onset of the inhibitory effects as well as the differences in potency observed between the (+) and (−) conformations of the drug. This interpretation is also consistent with the finding that latency to cessation of dopamine cell impulse flow following (+)-HA-966 was approximately twice as long as that associated with (−)-HA-966.

As previously reported (McMillen et al., 1992; Shepard and Lehmann, 1992), alterations in firing rate produced by (+)-HA-966 were accompanied by a marked increase in the regularity of firing as evidenced by a dose-dependent reduction in the coefficient of variation. Maximal effects were observed in response to a single dose of 30 mg/kg and led to the development of a pacemaker-like firing pattern similar to that observed spontaneously in vitro (Sanghera et al., 1984; Shepard and Bunney, 1988; Silva and Bunney, 1988). Phasic activity, in the form of spike doublets or multiple spike bursts, was totally suppressed by the drug. Evidence supporting the involvement of NMDA receptors in the expression of bursting activity (Johnson et al., 1992; Overton and Clark, 1992; Chergui et al.,

1993) together with the ability of (+)-HA-966 to antagonize these receptors in vivo, make it tempting to speculate that alterations in firing pattern following (+)-HA-966 occur as consequence of the blockade of a tonic excitatory drive onto dopamine cells in the substantia nigra. Support for this hypothesis derives from previous observations that kynurenic acid, a broad-spectrum excitatory amino acid receptor antagonist with preferential affinity for the glycine co-agonist binding site, is capable of suppressing bursting activity and increasing the regularity of dopamine cell firing (Grenhoff et al., 1988; Charlety et al., 1991). However, one of the principal findings of the present study is that low doses of (−)-HA-966, a compound with negligible affinity for the strychnine-insensitive glycine receptor (Pullan et al., 1990; Singh et al., 1990), are also capable of producing marked alterations in dopamine cell firing pattern. In fact, comparison of the magnitude of the changes in activity patterns produced by (+)- and (−)-HA-966 revealed the (−) enantiomer to be approximately 10 times more potent in both regularizing neuronal spike trains and suppressing phasic activity than the (+) enantiomer. These observations are consistent with previous results in which the pattern-regularizing effects of (+)-HA966 were found to persist following systemic administration of centrally active, competitive NMDA receptor antagonists (McMillen et al., 1992; Shepard et al., 1994) and suggest that the pattern-regularizing effects of (+)-HA-966 are not mediated by the drug's ability to displace glycine from its allosteric site on the NMDA receptor.

The similarity between the electrophysiological effects of (+)- and (−)-HA-966, together with the observation that the (−) enantiomer is the more potent of the two forms of the drug suggest that both compounds exert their effects through an interaction at the (−)-HA-966 binding site. Although neither compound exhibits appreciable affinity for $GABA_B$ receptors (McMillen et al., 1992; N. Bowery, personal communication), the electrophysiological changes produced by the enantiomers of (±)-HA-966 are qualitatively indistinguishable from those produced by systemic administration of baclofen and γ-hydroxybutyrate (Grace and Bunney, 1980b; Engberg and Nissbrandt, 1993; Engberg et al., 1993). Previous studies indicating that the neurochemical and electrophysiological effects of baclofen, γ-hydroxybutyrate and (−)-HA-966 can be blocked or reversed by $GABA_B$ receptor antagonists, have provided further support for the direct involvement of GABA receptors (Waldmeier, 1991; Engberg and Nissbrandt, 1993; Engberg et al., 1993; Shepard et al., 1993). The results of the present study indicating that the rate-suppressant effects of (+)-HA-966 can be fully reversed by CGP-35348 suggest that the inhibitory effects of this drug on mesencephalic dopamine neurons result from an interaction with $GABA_B$ receptors and provide additional support for the notion that the electrophysiological effects of the enantiomers of (±)-HA-966 are mediated through a common mode of action. Interestingly, neuronal firing pattern following CGP-35348-induced reversal of the rate-suppressant effects of (+)-HA-966 remained pacemaker-like suggesting that the inhibitory and pattern-regularizing effects of the drug are mediated by different pharmacological mechanisms. The observation that the inhibitory effects of (+)-HA-966, a drug with little or no direct affinity for $GABA_B$ receptors, can be rapidly reversed by CGP-35348 will require further study; however, these seemingly paradoxical findings are consistent with the notion that (+)-HA-966 may undergo metabolic conversion to an active conformation in vivo.

Identification and characterization of the cellular mechanism(s) mediating the unique electrophysiological effects of (±)-HA-966 may have important preclinical and clinical implications. As a potentially heuristic model of the pacemaker-like activity exhibited by dopamine cells in vitro, further study of the pharmacological properties of the drug may lead to additional insights regarding the mechanisms responsible for maintenance of normal activity patterns in vivo. The ability of (+)- and (−)-HA-966 to potently suppress irregular single spike and phasic activity and to induce a uniform pacemaker-like firing pattern among dopamine-containing neurons throughout the ventral midbrain may also provide a useful pharmacological tool to examine, in further detail, the influence of neuronal firing pattern on regulation of dopamine release (Nissbrandt et al., 1994). Confirmation of a functional interdependence between temporal coding and neurotransmitter release may form the basis of a novel strategy for reducing forebrain levels of dopamine without evoking the deleterious side effects associated with chronic blockade of postsynaptic dopamine receptors.

Acknowledgements

The authors gratefully acknowledge the generosity of Tocris Cookson, Ltd. in underwriting the expense of purchasing some of the drugs used in the present study. We would also like to thank Mrs. Sharon Candeloro for her assistance in preparing the manuscript. This work was supported by USPHS grant MH-48543.

References

Anthony, E.W. and M.E. Nevins, 1993, Anxiolytic-like effects of *N*-methyl-D-aspartate-associated glycine receptor ligands in the rat potentiated startle test, Eur. J. Pharmacol. 250, 317.

Bonta, I.L., C.J. De Vos and H. Grijsen, 1969a, 1-Hydroxy-3-amino-pyrrolidone-2 (HA-966). II. Extrapyramidal aspects, Arch. Int. Pharmacodyn. Ther. 182, 394.

Bonta, I.L., C.J. De Vos and F.C. Hillen, 1969b, 1-Hydroxy-3-amino-pyrrolidone-2 (HA-966). I. Behavior and motor effects, Arch. Int. Pharmacodyn. Ther. 182, 391.

Bonta, I.L., C.J. De Vos, H. Grijsen, F.C. Hillen, E.L. Noach and A.W. Sim, 1971, 1-Hydroxy-3-amino-pyrrolidone-2 (HA-966). A new GABA-like compound, with potential use in extrapyramidal diseases, Br. J. Pharmacol. 43, 514.

Broxterman, H.J. and J. Mos, 1980, Dopamine hypoactivity measured by in vivo voltammetry, Eur. J. Pharmacol. 68, 389.

Charlety, P.J., J. Grenhoff, K. Chergui, B. De La Chapelle, M. Buda, T.H. Svensson and G. Chouvet, 1991, Burst firing of mesencephalic dopamine neurons is inhibited by somatodendritic application of kynurenate, Acta Physiol. Scand. 142, 105.

Chergui, K., P.J. Charlety, H. Akaoka, C.F. Saunier, J.-L. Brunet, M. Buda, T.H. Svensson and G. Chouvet, 1993, Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo, Eur. J. Neurosci. 5, 137.

Corbett, R. and R.W. Dunn, 1991, Effects of HA-966 on conflict, social interaction, and plus maze behaviors, Drug Dev. Res. 24, 201.

Curtis, D.R., G.A.R. Johnston, C.G.A. Game and R.M. McCulloch, 1973, Antagonism of neuronal excitation by 1-hydroxy-3-amino-pyrrolidone-2, Brain Res. 49, 467.

Davies, J. and J.C. Watkins, 1972, Is 1-hydroxy-3-aminopyrrolidone-2 (HA-966) a selective excitatory amino acid antagonist?, Nature New Biol. 238, 61.

Davies, J. and J.C. Watkins, 1973, Microelectrophoretic studies on the depressant action of HA-966 on chemically and synaptically excited neurons in the cat cerebral cortex and cuneate nucleus, Brain Res. 59, 311.

De Lean, A., P.J. Munson and D. Rodbard, 1978, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves, Am. J. Physiol. 235, E97.

Diana, M., G. Mereu, A. Mura, F. Fadda, N. Passino and G. Gessa, 1991, Low doses of γ-hydroxybutyric acid stimulate the firing rate of dopaminergic neurons in unanesthetized rats, Brain Res. 566, 208.

Dunn, R.W., D.M. Flanagan, L.L. Martin, L.L. Kerman, A.T. Woods, F. Camacho, C.A. Wilmot, M.L. Cornfeldt, R.C. Effland, P.L. Wood and R. Corbett, 1992, Stereoselective *R*-(+) enantiomer of HA-966 displays anxiolytic effects in rodents, Eur. J. Pharmacol. 214, 207.

Engberg, G. and H. Nissbrandt, 1993, γ-Hydroxybutyric acid (GHBA) induces pacemaker activity and inhibition of substantia nigra dopamine neurons by activating $GABA_B$-receptors, Naunyn-Schmied. Arch. Pharmacol. 348, 491.

Engberg, G., T. Kling-Petersen and H. Nissbrandt, 1993, $GABA_B$-receptor activation alters the firing pattern of dopamine neurons in the rat substantia nigra, Synapse 15, 229.

Evans, R.H. and J.C. Watkins, 1978, Specific antagonism of excitant amino acids in the isolated spinal cord of the neonatal rat, Eur. J. Pharmacol. 50, 123.

Fletcher, E.J. and D. Lodge, 1988, Glycine reverses antagonism of *N*-methyl-D-aspartate (NMDA) by 1-hydroxy-3-aminopyrrolidone-2 (HA-966) but not by D-2-amino-5-phophonovalerate (D-AP5) on rat cortical slices, Eur. J. Pharmacol. 151, 161.

Foster, A.C. and J.A. Kemp, 1989, HA-966 antagonizes *N*-methyl-D-aspartate receptors through a selective interaction with the glycine modulatory site, J. Neurosci. 9, 2191.

French, E.D. and A. Ceci, 1990, Non-competitive *N*-methyl-D-aspartate antagonists are potent activators of ventral tegmental A10 dopamine neurons, Neurosci. Lett. 119, 159.

French, E.D., A. Mura and T. Wang, 1993, MK-801, Phencyclidine (PCP), and PCP-like drugs increase bursting firing in rat A10 dopamine neurons: comparison to competitive NMDA antagonists, Synapse 13, 108.

Gessa, G.L., I. Vargiu, F. Crabai, G.C. Boero, F. Caboni and R. Camba, 1966, Selective increase of brain dopamine induced by gamma-hydroxybutyrate, Life Sci. 5, 1921.

Goldstein, L.E., A.M. Rasmusson, B.S. Bunney and R.H. Roth, 1994, The NMDA glycine site antagonist (+)HA-966 selectively regulates conditioned stress-induced metabolic activation of the mesoprefrontal cortical dopamine but not serotonin systems: a behavioral, neuroendocrine, and neurochemical study in the rat, J. Neurosci. 14, 4937.

Gonon, F.G., 1988, Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry, Neuroscience 24, 19.

Grace, A.A. and B.S. Bunney, 1980a, Nigral dopamine neurons: intracellular recording and identification with L-dopa injection and histofluorescence, Science 210, 654.

Grace, A.A. and B.S. Bunney, 1980b, Effects of baclofen on nigral dopaminergic cell activity following acute and chronic haloperidol treatment, Brain Res. Bull. 5 (Suppl. 2), 537.

Grenhoff, J., C.-S. Tung and T.H. Svensson, 1988, The excitatory amino acid antagonist kynurenate induces pacemaker-like firing of dopamine neurons in rat ventral tegmental area in vivo, Acta Physiol. Scand. 134, 567.

Henderson, G., J.W. Johnson and P. Ascher, 1990, Competitive antagonists and partial agonists at the glycine modulatory site of the mouse *N*-methyl-D-aspartate receptor, J. Physiol. 430, 189.

Hillen, F.C. and E.L. Noach, 1971, The influence of 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) on dopamine metabolism in the rat corpus striatum, Eur. J. Pharmacol. 16, 222.

Hillen, F.C., A.W. Sim, E. Vincent, E.L. Noach and I.L. Bonta, 1969, 1-Hydroxy-3-amino-pyrrolidone-2 (HA-966). III. Neurochemical aspects, Arch. Int. Pharmacodyn. Ther. 182, 396.

Johnson, S.W., V. Seutin and R.A. North, 1992, Burst firing in dopamine neurons induced by *N*-methyl-D-aspartate: role of electrogenic sodium pump, Science 258, 665.

Manley, L.D., R. Kuczenski, D.S. Segal, S.J. Young and P.M. Groves, 1992, Effects of frequency and pattern of medial forebrain bundle stimulation on caudate dialysate dopamine and serotonin, J. Neurochem. 58, 1491.

Matthews, R.T., B.A. McMillen, S.G. Speciale, H. Jarrah, P.A. Shore, M.K. Sanghera, P.D. Shepard and D.C. German, 1984, Effects of zoxazolamine and related centrally acting muscle relaxants on nigrostriatal dopaminergic neurons, Brain Res. Bull. 12, 479.

McMillen, B.A., H.L. Williams, H. Lehmann and P.D. Shepard, 1992, On central muscle relaxants, strychnine-insensitive glycine receptors and two old drugs: zoxazolamine an HA-966, J. Neural Transm. 89, 11.

Menon, M.K., 1981, Demonstration of the antimyoclonic effect of 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) using a new animal model, Life Sci. 28, 2865.

Millan, M.J. and L. Seguin, 1993, (+)-HA-966, a partial agonist at the glycine site coupled to NMDA receptors, blocks formalin-induced pain in mice, Eur. J. Pharmacol. 238, 445.

Morrow, B.A., W.A. Clark and R.H. Roth, 1993, Stress activation of mesocorticolimbic dopamine neurons: effects of a glycine/NMDA receptor antagonist, Eur. J. Pharmacol. 238, 255.

Nissbrandt, H., A. Elverfors and G. Engberg, 1994, Pharmacologically induced cessation of burst activity in nigral dopamine neurons: significance for the terminal dopamine efflux, Synapse 17, 217.

Olpe, H.R., G. Karlsson, M.F. Pozza, F. Brugger, M. Steinmann, H. Riezen, G. Fagg, R.G. Hall, W. Froestl and H. Bittinger, 1990, CGP35348: a centrally active blocker of $GABA_B$ receptors, Eur. J. Pharmacol. 187, 27.

Overton, P. and D. Clark, 1992, Iontophoretically administered drugs acting at the *N*-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat, Synapse 10, 131.

Pullan, L.M., M. Britt, M.J. Chapdelaine, R.A. Keith, D. LaMonte, T.J. Mangano, J. Patel, R.J. Powell, R.J. Stumpo, P.J. Warwick, W.C. Zinkand and A.I. Salama, 1990, Stereoselectivity for the (*R*)-enantiomer of HA-966 (1-hydroxy-3-aminopyrrolidone-2) at the glycine site of the *N*-methyl-D-aspartate receptor complex, J. Neurochem. 55, 1346.

Roth, R.H. and N.J. Giarman, 1966, γ-Butyrolactone and γ-hydroxybutyric acid. I. Distribution and metabolism, Biochem. Pharmacol. 15, 1333.

Roth, R.H., J.D. Doherty and J.R. Walters, 1980, Gamma-hydroxybutyrate: a role in the regulation of central dopaminergic neurons?, Brain Res. 189, 556.

Sanghera, M.K., M.E. Trulson and D.C. German, 1984, Electrophysiological properties of mouse dopamine neurons: in vivo and in vitro studies, Neuroscience 12, 793.

Shepard, P.D. and B.S. Bunney, 1988, Effects of apamin on the discharge properties of putative dopamine-containing neurons in vitro, Brain Res. 463, 380.

Shepard, P.D. and H. Lehmann, 1992, (±)-1-Hydroxy-3-aminopyrrolidone-2 (HA-966) inhibits the activity of substantia nigra dopamine neurons through a non-*N*-methyl-D-aspartate receptor-mediated mechanism, J. Pharmacol. Exp. Ther. 261, 387.

Shepard, P.D., S.T. Connelly and K.C. Grobaski, 1993, Electrophysiological effects of (−)-HA-966: evidence for an interaction with $GABA_B$ receptors, Soc. Neurosci. Abstr. 19, 1372.

Shepard, P.D., K.C. Grobaski and S.T. Connelly, 1994, The cellular basis of conditional bursting activity in mesencephalic dopamine-containing neurons, Soc. Neurosci. Abstr. 20, 12.

Silva, N.L. and B.S. Bunney, 1988, Intracellular studies of dopamine neurons in vitro: pacemakers modulated by dopamine, Eur. J. Pharmacol. 149, 307.

Singh, L., A.E. Donald, A.C. Foster, P.H. Hutson, L.L. Iversen, S.D., Iversen, J.A. Kemp, P.D. Leeson, G.R. Marshall, R.J. Oles, T. Priestly, L. Thorn, M.D. Tricklebank, C.A. Vass and B.J. Williams, 1990, Enantiomers of HA-966 (3-amino-1-hydroxypyrrolid-2-one) exhibit distinct central nervous system effects: (+)-HA-966 is a selective glycine/*N*-methyl-D-aspartate receptor antagonist, but (−)-HA-966 is a potent γ-butyrolactone-like sedative, Proc. Natl. Acad. Sci. USA 87, 347.

Stone, T.W., 1976, Blockade by amino acid antagonists of neuronal excitation mediated by the pyramidal tract, J. Physiol. 257, 187.

Suaud-Chagny, M.F., K. Chergui, G. Chouvet and F. Ganon, 1992, Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area, Neuroscience 49, 63.

Tung, C.S., J. Grenhoff and T.H. Svensson, 1991, Kynurenate blocks the acute effects of haloperidol on midbrain dopamine neurons recorded in vivo, J. Neural Transm. 84, 53.

Van der Velden, K.H.M., 1969, 1-Hydroxy-3-amino-pyrrolidone-2 (HA-966). IV. Clinical aspects, Arch. Int. Pharmacodyn. Ther. 182, 398.

Van Valkenburg, C.F.M. and E.L. Noach, 1978, Electrical stimulation of the nigrostriatal pathway after HA-966 block of striatal dopamine release, Eur. J. Pharmacol. 48, 171.

Vartanian, M.G. and C.P. Taylor, 1991, Different stereoselectivity of the enantiomers of HA-966 (3-amino-1-hydroxy-2-pyrrolidinone) for neuroprotective and anticonvulsant actions in vivo, Neurosci. Lett. 133, 109.

Waldmeier, P.C., 1991, The $GABA_B$ antagonist, CGP 35348, antagonizes the effects of baclofen, γ-butyrolactone and HA 966 on rat striatal dopamine synthesis, Naunyn-Schmied. Arch. Pharmacol. 343, 173.

Walters, J.R., G.K. Aghajanian and R.H. Roth, 1972, Dopaminergic neurons: inhibition of firing by γ-hydroxybutyrate, Proceedings Fifth International Congress Pharmacology 600, 100.

Werner, G. and V.B. Mountcastle, 1963, The variability of central neural activity in a sensory system, and its implications for the central reflection of sensory events, J. Neurophysiol. 26, 958.

White, W.F., J.V. Nadler and C.W. Cotman, 1979, The effect of acidic amino acid antagonists on synaptic transmission in the hippocampal formation in vitro, Brain Res. 164, 177.