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Metabotropic Glutamate Receptors Mediate Excitatory Transmission in the Nucleus of the Solitary Tract

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Following microinjection into the nucleus tractus solitarius (NTS), the effects of glutamate on the baroreceptor reflex are poorly antagonized by kynurenic acid and DL-2-amino-5-phosphonovaleric acid, suggesting the possible involvement of metabotropic glutamate receptors in this response. The metabotropic glutamate receptor agonist 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) depolarized neurons located medial to the tractus solitarius (TS) at the level of the area postrema in coronal sections of the rat NTS. This effect was mimicked by glutamate and was not blocked by antagonists at α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate or NMDA receptors. 1*S*,3*R*-ACPD also produced an inward current under voltage clamp that was not accompanied by a rise in $[Ca^{2+}]_i$, monitored with the CA^{2+}-sensitive dye fura-2. Conversely, the muscarinic agonist carbachol produced an outward current and a rise in $[Ca^{2+}]_i$. 1*S*,3*R*-ACPD reduced both the excitatory and the inhibitory postsynaptic current resulting from single electrical stimuli in the region of the TS. High-frequency stimulation of the TS produced an inward current in the presence of AMPA/kainate and NMDA receptor blockers. This current had similar properties to that produced by 1*S*,3*R*-ACPD. Thus, metabotropic glutamate receptors may mediate a component of excitatory transmission in the NTS.

The actions of glutamate (GLU) at α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate and NMDA receptors have been shown to be responsible for producing fast excitatory synaptic transmission at numerous synapses in the CNS. The properties of these receptors have become increasingly well understood over the last few years and have been shown to mediate many of the physiological and pathological effects of GLU in the brain (Mayer and Westbrook, 1987). However, not all GLU receptors are ligand-gated ion channels. A third type of GLU receptor also exists that is linked to one or more G-proteins and effector systems. This receptor has sometimes been described as the "metabotropic" GLU receptor (Sugiyama et al., 1989; Schoepp et al., 1990). Molecular biological studies have revealed that this protein has a sequence related to other members of the heptahelical G-protein–linked receptor superfamily (Houamed et al., 1991; Masu et al., 1991). The initial effect associated with metabotropic GLU receptor activation was stimulation of phospholipase C in both neurons and astrocytes, resulting in the synthesis of inositol trisphosphate (IP_3) and mobilization of Ca^{2+} from intracellular stores (Schoepp et al., 1990; reviewed in Miller, 1991). Although metabotropic GLU receptors are widely distributed in the CNS (Horikoshi et al., 1989), the neurophysiological consequences of metabotropic receptor activation are only now becoming evident (Miller, 1991). One reason for this has been the recent introduction of 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD, or *trans*-ACPD), which is a rather selective agonist at such receptors (Palmer et al., 1989; Manzoni et al., 1990). Thus, under the appropriate circumstances, the effects of 1*S*,3*R*-ACPD can be taken as indicating the involvement of metabotropic GLU receptors. Initial experiments have shown that 1*S*,3*R*-ACPD can produce a variety of electrophysiological effects, including slow depolarization (Stratton et al., 1989, 1990; Charpak et al., 1990; Charpak and Gähwiler, 1991; Zheng and Gallagher, 1991), inhibition of spike accommodation (Stratton et al., 1989, 1990; Baskys et al., 1990a,b; Charpak et al., 1990; Charpak and Gähwiler, 1991), and presynaptic inhibition (Baskys and Malenka, 1991; Desai and Conn, 1991; Lovinger, 1991; Pacelli and Kelso, 1991). However, many questions remain to be addressed. For example, what is the complete repertoire of cellular effects produced by metabotropic receptors, and when are such receptors activated by synaptically released GLU?

In order to answer such questions, our attention was drawn to the nucleus of the solitary tract (NTS). The NTS is the primary medullary region for the coordination of a variety of cardiovascular, respiratory, and visceral reflexes. Neurons within the rostrocaudal subdivision of the NTS, adjacent to the area postrema and medial to the tractus solitarius (TS), have been shown to affect baroreceptor (Leone and Gordon, 1989) and Breuer–Hering (Bonham and McCrimmon, 1990) reflexes. GLU appears to be the primary neurotransmitter used by afferent projections to the NTS from the periphery (Meeley et al., 1989). Furthermore, agonists acting at AMPA/kainate or NMDA receptors appear to activate the majority of neurons within the NTS (Drewe et al., 1990). When microinjected into the NTS *in vivo*, the GLU receptor antagonist kynurenate has been shown to inhibit the baroreceptor reflex, as well as the hypotensive and bradycardic effects of injected kainate or NMDA (Talman, 1989). Curiously, however, the effects of exogenous GLU or quisqualate were poorly inhibited by kynurenate (Leone and Gordon, 1989; Talman, 1989). We took this observation to indicate

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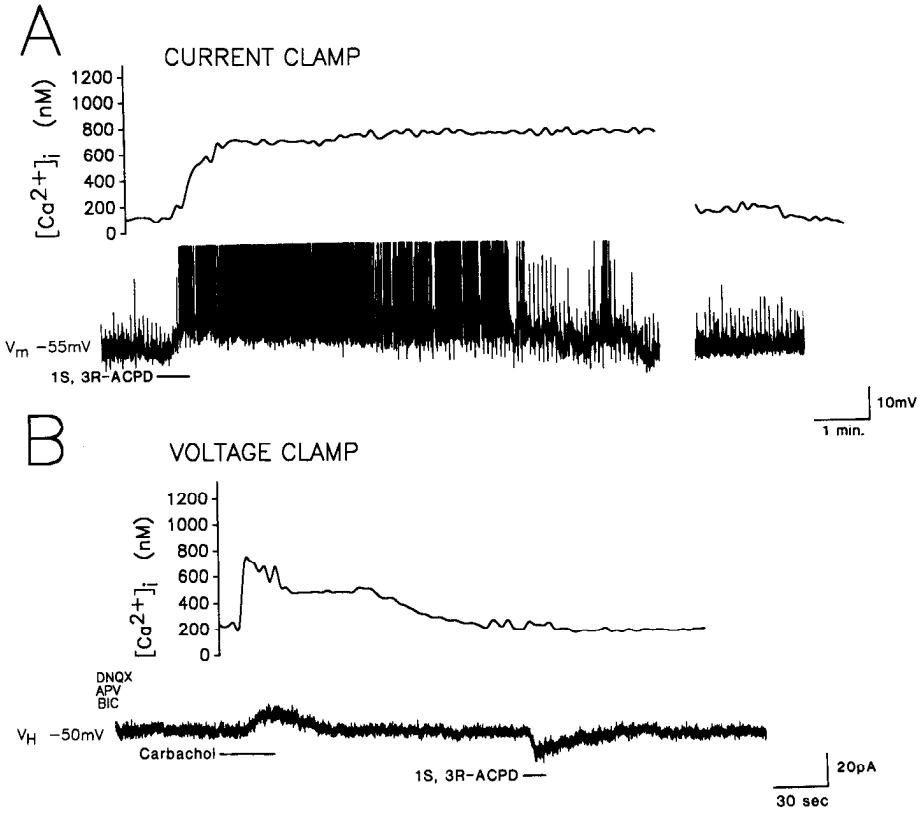

Figure 1. Representative voltage, current, and $[Ca^{2+}]_i$ measurements from an NTS neuron exposed to 1*S*,3*R*-ACPD. *A*, Acute exposure to 50 μM 1*S*,3*R*-ACPD in the presence of the $GABA_A$ antagonist BIC (10 μM) produced long-lasting depolarization and an increase in somatic $[Ca^{2+}]_i$ in NTS neurons. Resting V_m at the start of the trace ≈ −50 mV. The break in the graph encompasses 4 min when $[Ca^{2+}]_i$ and V_m slowly decayed back to resting levels. *B*, The neuron in *A* was voltage clamped at its resting V_m in the presence of the GLU receptor antagonists DNQX (1 μM), APV (50 μM), and BIC (10 μM). The muscarinic agonist carbachol (10 μM) produced an outward current and an increase in $[Ca^{2+}]_i$. 1*S*,3*R*-ACPD (50 μM) produced an inward current with no change in $[Ca^{2+}]_i$.

that some of the actions of GLU in this nucleus might be mediated by additional types of GLU receptors. In the present study we have examined the response of neurons within the NTS to application of 1*S*,3*R*-ACPD utilizing whole-cell patch recording in coronal brainstem slices. In order to be able to define the relationship between the effects observed and those previously reported on $[Ca^{2+}]_i$, these measurements were combined with simultaneous monitoring of $[Ca^{2+}]_i$ in the same cells. In addition, the effects of 1*S*,3*R*-ACPD on electrically evoked neurotransmitter release were also assessed. We report that 1*S*,3*R*-ACPD produces both pre- and postsynaptic responses within the NTS that may be of significance for cardiovascular reflexes *in vivo*.

Materials and Methods

Electrophysiology. Sprague–Dawley rats of either sex, aged between 4 and 6 weeks, were given overdoses of ether, and the whole brain, including the cervical spinal cord, was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF), which contained (in mM) NaCl, 125; $NaHCO_3$, 26.2; NaH_2PO_4, 1; KCl, 3; $MgSO_4$, 1.5; $CaCl_2$, 2.5; and glucose, 10. The final osmolarity of aCSF was between 315 and 330 mOsm. Transverse 300 μm slices containing the NTS were prepared and placed in a holding chamber filled with 23°C aCSF and continuously bubbled with a 95%/5% mixture of O_2/CO_2. Following a 1 hr recovery, a single slice was transferred to the recording chamber (volume of 1 ml), where it was submerged and held in place between two nets and continuously perfused (4–5 ml/min) with oxygenated aCSF at room temperature.

Patch electrodes were filled with a solution containing (in mM) K^+ gluconate, 145; $MgCl_2$, 2; HEPES, 5; fura-2 pentapotassium salt, 0.1; and K_2-ATP, 5. Pipette solutions were adjusted to a pH of 7.2 with KOH and had an osmolarity of 305–315 mOsm. Electrode resistance was between 2 and 5 MΩ. Neurons were approached under visual inspection of the slice at 400× magnification with Hoffman modified optics. Seals in excess of 5 GΩ were obtained and background fluorescence determined immediately prior to whole-cell recording. Recordings were obtained from cells with stable membrane potentials (V_m) greater than −50 mV, overshooting action potentials, and access resistance <25 MΩ. Recordings were made with an Axoclamp 2A amplifier (Axon Instruments) in discontinuous single-electrode current or voltage clamp, with switching frequencies that allowed for complete settling of the headstage signal (4–14 kHz).

Synaptic stimulation was carried out by placing a bipolar tungsten stimulating electrode in the region of the ipsilateral tractus solitarius (TS). Single, subthreshold stimuli were delivered (5–200 μsec, 1–30 V) at 5–10 sec intervals. In the majority of cells (~75%), in which evidence of both evoked IPSPs and EPSPs was observed, EPSPs were pharmacologically isolated by the addition of the $GABA_A$ antagonist bicuculline (BIC; 10 μM). Unless otherwise indicated, the duration and intensity of synaptic stimulation were then adjusted to attain highly reproducible responses. Evoked IPSPs were similarly isolated by the addition of the GLU receptor antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX; 1 μM) and DL-2-amino-5-phosphonovaleric acid (APV; 50 μM). The combined application of antagonists blocked all discernable evoked synaptic activity, even at stimulus intensities 10× the control value. All drugs were applied in the perfusate. The osmolarity of all drug-containing aCSF solutions was found to be within the range of the control aCSF. A delay in the change of bath solutions inherent to the perfusion system was estimated between 15 and 30 sec. Figures indicating the application of drugs have been corrected for this delay.

Data were filtered at 3 kHz, stored on a chart recorder (Gould), digitized at 125 kHz by a computer-driven analogue-to-digital converter (INDEC Systems), and stored on hard disk for subsequent analysis.

The peak amplitude of EPSPs and IPSPs was quantitated following pharmacological isolation by the addition of BIC or DNQX/APV, respectively. Comparisons were made between the average peak amplitude of five stimuli immediately prior to the application of drugs and the average of five values obtained at the apparent peak of the response.

Under conditions of voltage clamp, evoked excitatory (EPSCs) and inhibitory (IPSCs) postsynaptic currents were pharmacologically isolated and averaged as before at holding potentials (V_h) between −50 and −65 mV.

Pooled data on evoked events were obtained from three or more neurons and expressed as the mean ± SEM.

$[Ca^{2+}]_i$ measurements. For fura-2 epifluorescence measurements, light from an Xe lamp dual monochromator light source (PTI) was split by

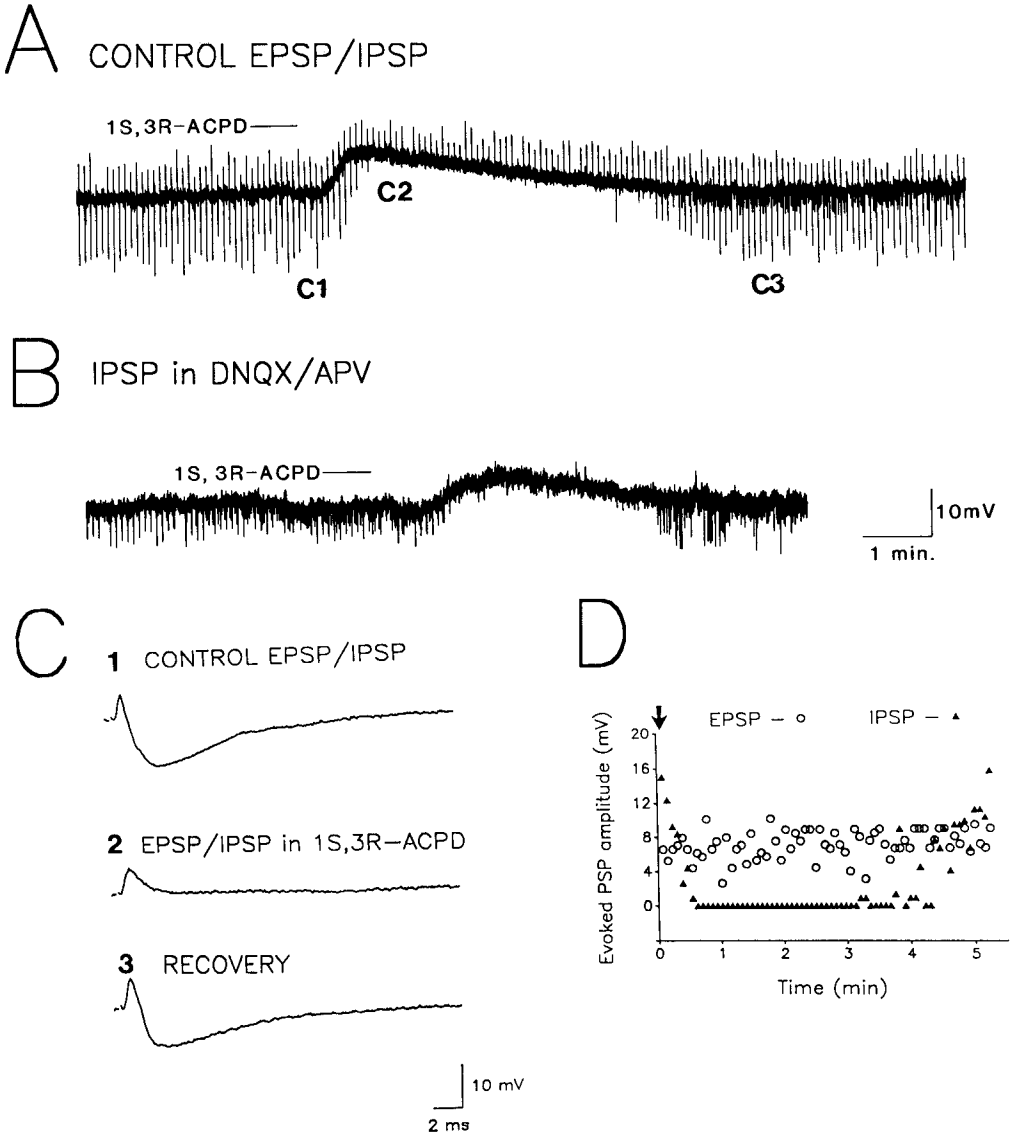

Figure 2. Depolarizing responses and depression of evoked synaptic transmission recorded in an NTS neuron. *A*, Bath application of 1*S*,3*R*-ACPD (50 μM) produced a slow depolarization of this neuron and a reduction in IPSPs (*downward deflections* on trace) evoked by single subthreshold electrical stimulation in the region of the tractus solitarius (1 × 5 sec). Evoked EPSPs (*upward deflections*) were unaffected by 1*S*,3*R*-ACPD in this neuron. In *B*, the pre- and postsynaptic effects of 1*S*,3*R*-ACPD on the cell in *A* were preserved in the presence of the ionotropic GLU receptor antagonists DNQX (1 μM) and APV (50 μM). Although the stimulus strength was unchanged from *A*, note the decrease in the IPSP amplitude. This reflects the mixed direct (monosynaptic) and indirect (polysynaptic, DNQX/APV-sensitive) driving force underlying the evoked IPSP. Resting V_m at the start of *A* and *B* was −61 mV. *C*, Averaged (five stimuli), higher-time-resolution traces of the evoked EPSP/IPSP complex and the effect of 1*S*,3*R*-ACPD taken from the cell in *A* at points *C1*, *C2*, and *C3*. Note the broadening of the EPSP (*2*) during the block of the IPSP by 1*S*,3*R*-ACPD. Similar observations following the addition of BIC suggest that, under control conditions, the short latency of the IPSP curtails the monosynaptic EPSP. *D*, Plot of peak evoked PSP amplitude for each evoked EPSP/IPSP complex, taken from cell in *A* immediately following the addition of 1*S*,3*R*-ACPD (*arrow*).

means of a computer-driven chopper into 340 and 380 nm excitation wavelengths (4 nm bandwidth). The collminated beam was passed through a Zeiss 40× water immersion objective mounted on a Leitz upright microscope, with emitted fluorescence from a 10–40 μm^2 area encompassing the soma passed through a 480 nm longpass filter into a photomultiplier tube (Thorn/EMI). Synchronized digital conversion (125 kHz) of the analog signal (8 msec/wavelength) was performed by a software package adapted by S. Murphy from previously described software (Murphy and Miller, 1988). Calculation of $[Ca^{2+}]_i$ was performed by the method of Grynkiewicz et al. (1985), with calibrations made as previously described (Murphy and Miller, 1988).

Results

1*S*,3*R*-ACPD produced several effects on neurons in the NTS. The first of these is illustrated in Figure 1. Concentrations of 1*S*,3*R*-ACPD between 5 and 50 μM depolarized neurons, sometimes bringing them to the threshold for firing action potentials. It can be seen that accompanying this response there was a large rise in $[Ca^{2+}]_i$, from a basal level of 128.3 ± 16.0 nM to a peak level of 800.2 ± 58.9 nM (n = 6). Following washout of the 1*S*,3*R*-ACPD, the response gradually subsided. In particular, the $[Ca^{2+}]_i$ usually took 3–5 min to return to baseline levels (Fig. 1*A*). In cells that depolarized but did not reach action potential threshold, no increase in $[Ca^{2+}]_i$ was noted (basal $[Ca^{2+}]_i$ = 135.7 ± 10.1 nM; in the presence of 50 μM 1*S*,3*R*-ACPD, $[Ca^{2+}]_i$ = 155.3 ± 17.3 nM; n = 7). The mean depolarization following acute application of 50 μM 1*S*,3*R*-ACPD to neurons examined under current-clamp conditions was 5.8 ± 0.8 mV (n = 13), from a resting V_m between −63 and −52 mV.

When neurons were voltage clamped near their resting membrane potentials (−50 to −60 mV) in the presence of DNQX, APV, and BIC (Fig. 1*B*), 1*S*,3*R*-ACPD produced an inward current that appeared to be independent of a rise in the somatic $[Ca^{2+}]_i$ (basal $[Ca^{2+}]_i$ = 117.7 ± 32.6 nM; in the presence of 50 μM 1*S*,3*R*-ACPD, $[Ca^{2+}]_i$ = 128.2 ± 5.3 nM; n = 7). In contrast, the muscarinic agonist carbachol produced an outward current that was preceded by a robust rise in $[Ca^{2+}]_i$ (basal $[Ca^{2+}]_i$ = 192.0 ± 9.7 nM; in the presence of 10 μM carbachol, peak $[Ca^{2+}]_i$ = 985.7 ± 136.4 nM; n = 3). In these experiments the lack of effect of 1*S*,3*R*-ACPD on $[Ca^{2+}]_i$ was not due to a prior depletion of intracellular Ca^{2+} stores following muscarinic receptor activation, as the same result was also obtained when 1*S*,3*R*-ACPD was applied to cells prior to other stimuli (e.g., Fig. 4*C*).

In addition to producing postsynaptic depolarization, 1*S*,3*R*-ACPD also reduced both components of the evoked glutama-

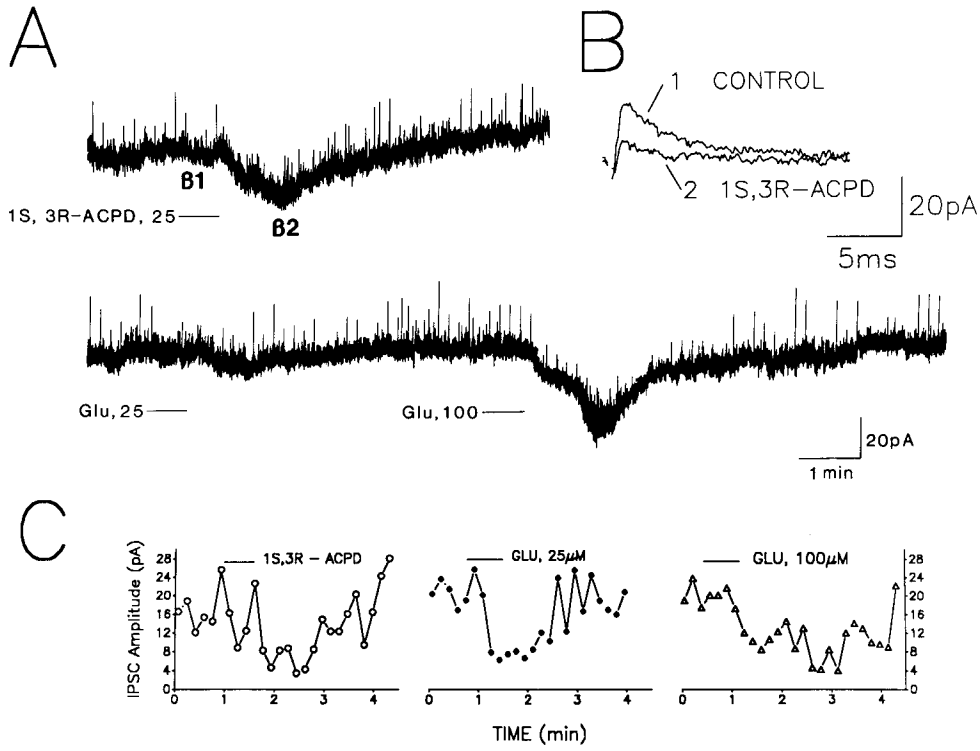

Figure 3. Pre- and postsynaptic effects of 1*S*,3*R*-ACPD were reproduced by exogenous GLU. *A*, Continuous chart record of a current trace following sequential exposure to 1*S*,3*R*-ACPD (25 μM) and GLU (25 and 100 μM) in the presence of 1 μM DNQX and 50 μM APV. Cell was clamped at −60 mV. IPSCs (*upward deflections* on trace) were evoked by electrical (1 × 10 sec) stimulation in the region of the TS. *B*, Higher-time-resolution, averaged (n = 5) traces of the evoked IPSC, taken from cell in *A* at points *B1* and *B2*, illustrating control and peak inhibition by 1*S*,3*R*-ACPD. *C*, Plot of 1*S*,3*R*-ACPD and GLU effects on individual IPSC amplitude from cell in *A*.

tergic EPSP/GABAergic IPSP complex produced by a single subthreshold stimulus in the region of the TS (Fig. 2). Note the broadening of the EPSP during the block of the IPSP by 1*S*,3*R*-ACPD. Similar observations using BIC suggest that the short latency of the IPSP curtails the preceding EPSP. Thus, in order to evaluate precisely the effects of 1*S*,3*R*-ACPD on evoked synaptic activity, the relative contributions of GABA or GLU were first eliminated by pharmacological means. In the example illustrated in Figure 2, the EPSP (upward deflection on trace) was eliminated by the addition of the glutamate receptor antagonists DNQX and APV, following the evoked IPSP (downward deflection on trace) to be clearly observed. The decrease in the amplitude of the IPSP in the presence of DNQX/APV (compare Fig. 2*A*,*B*) reflects the mixed direct (monosynaptic) and indirect (polysynaptic, AMPA/NMDA receptor–mediated) nature of inhibitory events driven by afferent inputs to the TS.

Table 1. Percentage change in peak evoked PSP or PSC amplitude produced by 50 μM 1*S*,3*R*-ACPD

	Mean ± SE (%)	n
Current clamp		
EPSPs	−67.1 ± 5.9	20
IPSPs	−95.5 ± 2.6	6
Voltage clamp		
EPSCs	−53.0 ± 6.6	12
IPSCs	−92.2 ± 4.4	8

Synaptic currents or potentials were evoked by subthreshold electrical stimuli in the region of the tractus solitarius. Excitatory activity was recorded in the presence of BIC (10 μM). Inhibitory activity was recorded in the presence of DNQX (1 μM) and APV (50 μM). The amplitudes of five consecutive synaptic events were averaged, normalized to 100%, and compared to the averaged amplitude at the peak of the 1*S*,3*R*-ACPD response. Individual percentage inhibitory effects were pooled. Synaptic potentials were recorded at the resting V_m. Synaptic currents were recorded at V_h between −50 and 60 mV.

Although 1*S*,3*R*-ACPD was observed to block the remaining monosynaptic IPSP completely in virtually every instance, this was not so for monosynaptic EPSPs measured in the presence of BIC, where inhibition was variable and less pronounced (see below). Both the postsynaptic inward current and presynaptic inhibitory effect on evoked IPSP/Cs produced by 1*S*,3*R*-ACPD were also observed following the application of GLU in the presence of DNQX and APV as shown in Figure 3. The peak inhibition by 25 μM GLU was similar to that of 100 μM GLU, possibly indicating that the effectiveness of GLU was limited by uptake systems preserved in the slice.

The effects of 1*S*,3*R*-ACPD on evoked postsynaptic potentials (PSPs) and currents (PSCs) measured under current- and voltage-clamp conditions, respectively, are summarized in Table 1. The higher SE obtained for 1*S*,3*R*-ACPD effects on pharmacologically isolated evoked excitatory events reflects the greater variability of inhibition observed in these experiments. Although the majority of neurons (56 out of 61) responded to 1*S*,3*R*-ACPD as detailed above, excitatory transmission was completely inhibited in two cells without a discernable inward current, as illustrated in Figure 4*A*. Furthermore, an additional three cells responded with an inward current but without significant inhibition of the EPSC evaluated in the presence of BIC (Fig. 4*C*).

In order to examine further the nature of the postsynaptic response to 1*S*,3*R*-ACPD, whole-cell *I*/*V* curves in the presence of synaptic blockers were generated under control conditions and during the peak of inward current, as illustrated in Figure 5. Neurons exposed to 1*S*,3*R*-ACPD exhibited an inward current associated with a decrease in the slope conductance of the *I*/*V* relationship. Because the 1*S*,3*R*-ACPD current was relatively small (<50 pA) at potentials near the resting potential and appeared to reverse in the region of strong rectification, an alternative method was used to estimate the reversal potential

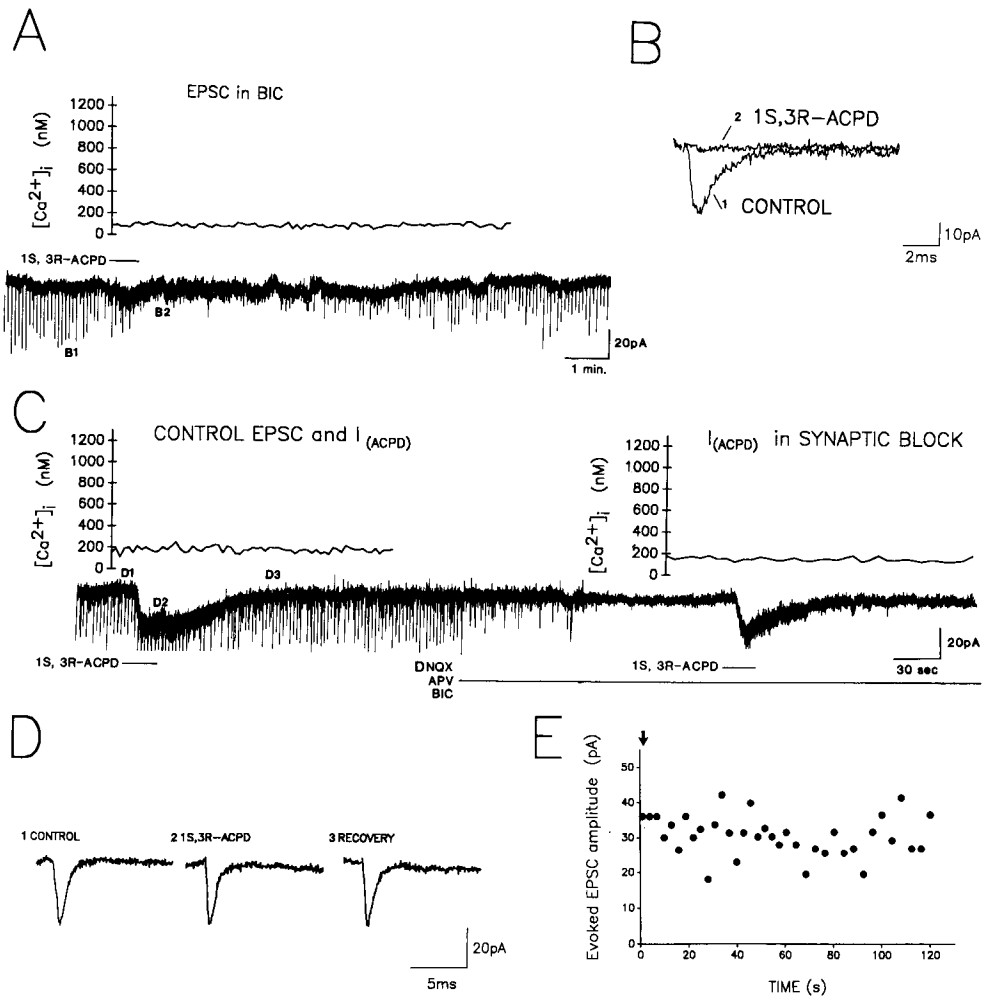

Figure 4. Pre- and postsynaptic 1*S*,3*R*-ACPD responses were observed independently in a few NTS neurons. *A*, Inhibition of evoked EPSCs (1 × 5 sec) by 50 μM 1*S*,3*R*-ACPD in the absence of an apparent postsynaptic inward current. The neuron was clamped at −55 mV in the presence of BIC (10 μM). *B*, Higher time resolution of averaged EPSCs ($n = 5$) from neuron in *A* at points *B1* and *B2*, illustrating control and peak inhibitory effect by 1*S*,3*R*-ACPD. *C*, Sequential exposure to 50 μM 1*S*,3*R*-ACPD in the absence (*left*) and presence (*right*) of synaptic blockers (DNQX, 1 μM/APV, 50 μM/BIC, 10 μM) illustrating the inward current (I_{ACPD}) and absence of a change in $[Ca^{2+}]_i$. Note the decrease in width of the chart recorder trace in the presence of synaptic blockers, reflecting inhibition of spontaneous synaptic currents. Although evoked EPSCs were cut off by the chart record, averaged ($n = 5$) higher-time-resolution traces in *D* taken at points *D1*, *D2*, and *D3*, and the plot of individual EPSC amplitude in *E* show that the evoked EPSC was not affected by 1*S*,3*R*-ACPD. The *arrow* in *E* indicates the onset of 1*S*,3*R*-ACPD application. Similar results were obtained in two other cells.

for the current. Cells were clamped at potentials between −90 and 0 mV, and the peak current was obtained following 30 sec bath application of 1*S*,3*R*-ACPD applied at 15 min intervals. Cells were returned to the control (−50 mV test potential) and exposed to 1*S*,3*R*-ACPD between exposures at the other holding potentials to address the question of desensitization. The peak current at the control holding potential varied by <5% using this paradigm, suggesting an absence of strong desensitization. The pooled results of this series of experiments are illustrated in Figure 5*B*. The estimated reversal potential for the 1*S*,3*R*-ACPD induced current was ~ −75 mV.

The current produced by 1*S*,3*R*-ACPD was occluded by Ba^{2+} (1–5 mM; $n = 5$). Thus, while Ba^{2+} itself produced an inward current, no additional current was evident following the combined application of Ba^{2+} and 1*S*,3*R*-ACPD, as illustrated in Figure 5*C*. As the *I*/*V* curves for Ba^{2+}, 1*S*,3*R*-ACPD, or a combination of the two were identical, both 1*S*,3*R*-ACPD and Ba^{2+} seemed to be reducing a similar membrane conductance.

The effects of 1*S*,3*R*-ACPD (5 μM) were not blocked by 2-amino-3-phosphonoproprionic (L-AP3; 50 μM; $n = 5$). This compound has been shown to inhibit agonist actions at metabotropic glutamate receptors in some instances (Palmer et al., 1989; Schoepp and Johnson, 1989; Schoepp et al., 1990). Higher concentrations of L-AP3 induced nonspecific membrane effects and could not therefore be used. The addition of the K^+ channel blocker Cs^+ (5 mM) or the isosmotic substitution of 25 mM tetraethylammonium (TEA) for part of the extracellular Na^+ also failed to block the 1*S*,3*R*-ACPD (50 μM)-induced current in these cells ($n = 3$ each).

In order to determine whether synaptically released GLU could activate postsynaptic metabotropic responses in the NTS, stimulus trains of increasing frequency and duration were applied to the region of the TS. Stimulus intensity was first established at a level sufficient to evoke a subthreshold, monosynaptic EPSP/C in the absence of synaptic blockers. Slices were then perfused with DNQX/APV/BIC. No residual PSP or PSC was observed following single stimuli at the frequencies (0.1–0.2 Hz) used in the previously detailed experiments. Stimulus frequency and train duration were then alternately increased until discernable current or voltage responses were obtained. Following this paradigm, the most sensitive cell ($n = 11$) produced a 10 pA inward current from a $V_h = -50$ mV after a 100 msec, 20 Hz stimulus train. Peak inward current (18 pA) was obtained with a 300 msec, 50 Hz train. In the remaining neurons, stimulus trains of >100 msec, >20 Hz were necessary to observe postsynaptic responses reminiscent of those produced by exogenously applied 1*S*,3*R*-ACPD. The reversal potential of the inward current produced was −73 ± 3 mV ($n = 3$), similar to that produced by 1*S*,3*R*-ACPD itself. Furthermore, the inward current was not accompanied by a rise in $[Ca^{2+}]_i$. The synaptically mediated postsynaptic response was occluded following the addition of 1–5 mM Ba^{2+} ($n = 5$), similar to the findings with 1*S*,3*R*-ACPD itself (Fig. 6*A*). Postsynaptic responses to high-frequency stimulation were also occluded in the continuous

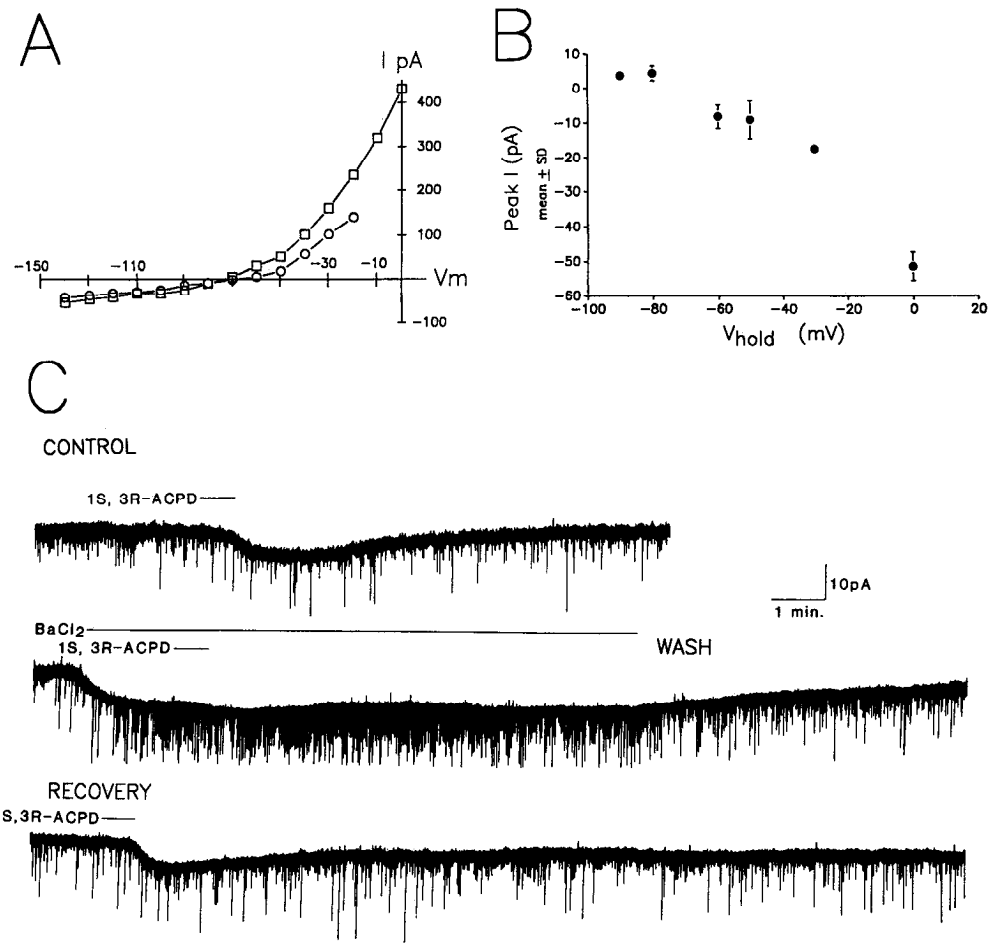

Figure 5. Change in the current/voltage relationship for NTS neurons by 1*S*,3*R*-ACPD. *A*, Control *I*/*V* curve (□) was measured from a holding potential of −55 mV immediately prior to application of 1*S*,3*R*-ACPD (50 μM). 1*S*,3*R*-ACPD (○) reduced the slope conductance of the *I*/*V* relationship throughout the range of test potentials. Because the postsynaptic ACPD current was always small (<50 pA) at potentials near the resting V_m of NTS neurons, peak 1*S*,3*R*-ACPD currents obtained at various holding potentials following repeated exposure to 1*S*,3*R*-ACPD (see Results) were pooled as shown in *B*, which illustrates pooled data from five cells following repeat exposure to 1*S*,3*R*-ACPD. The estimated reversal potential for the postsynaptic 1*S*,3*R*-ACPD current was −75 mV. Where absent, the SD (*error bars*) at a given holding potential was less than the width of the data point. *C*, Voltage-clamp recording (V_h = −50 mV) illustrating that the inward current produced by 1*S*,3*R*-ACPD was reversibly occluded by the addition of 5 mM Ba^{2+}. Thus, while Ba^{2+} itself produced an inward current in NTS neurons, 1*S*,3*R*-ACPD failed to induce a further inward current in the presence of Ba^{2+}.

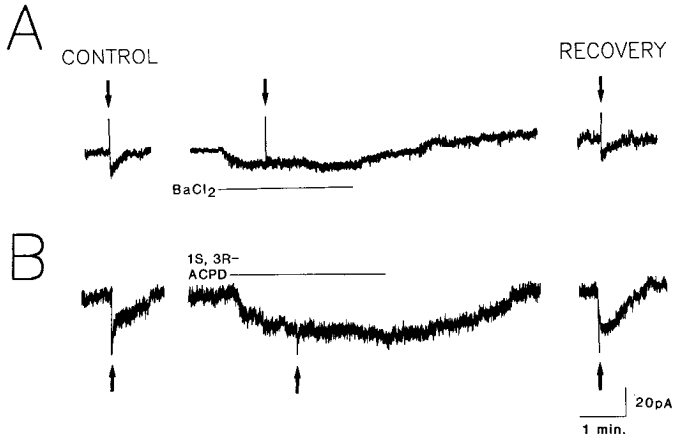

Figure 6. Voltage-clamp recordings of NTS neurons following high-frequency stimulation of the TS. *A*, Control inward current evoked by high-frequency stimulation (*first arrow*) of the TS (300 msec, 50 Hz, 3 V) in the presence of DNQX (1 μM), APV (50 μM), and BIC (10 μM). Following the addition of 5 mM $BaCl_2$, an inward current was observed. However, no additional inward current followed an identical, high-frequency stimulation of the TS (*second arrow*). The stimulus-induced inward current recovered following Ba^{2+} washout. *B*, Control inward current in another NTS neuron evoked by a high-frequency (500 msec, 50 Hz, 4 V) stimulus train (*first arrow*) in the presence of synaptic blockers as in *A*. The addition of 1*S*,3*R*-ACPD produced an inward current that occluded further postsynaptic responses to an identical stimulus train (*second arrow*). Stimulus-induced current recovered following washout of 1*S*,3*R*-ACPD. V_h in *A* and *B* was −60 mV.

presence of 1*S*,3*R*-ACPD itself (Fig. 6*B*; n = 5), supporting the notion that synaptically released GLU may be activating postsynaptic metabotropic receptors. To confirm that high-frequency stimulation was not directly activating the postsynaptic cell by electronic spread through the slice, synaptic responses were blocked by the addition of TTX (1 μM). TTX completely inhibited postsynaptic voltage or current responses to high-frequency stimulation in all cases.

Discussion

In the present study we have demonstrated that activation of metabotropic GLU receptors using 1*S*,3*R*-ACPD produces both excitatory postsynaptic and inhibitory presynaptic effects within a region of the NTS known to contain a high density of cardiac afferent projections (Donoghue et al., 1985). Such results are in keeping with a growing number of observations on synaptic events in the CNS which seem to be mediated by metabotropic GLU receptors (reviewed in Miller, 1991). These include a role for these receptors in the induction of long-term depression in the cerebellum (Kano et al., 1988; Linden et al., 1991), in producing long-term potentiation in the hippocampus (McGuinness et al., 1991), and in producing slow excitation of CA3 hippocampal pyramidal neurons following stimulation of mossy fibers (Charpak and Gähwiler, 1991).

In addition to the effects of exogeneously applied 1*S*,3*R*-

ACPD, we provide evidence that postsynaptic mechanisms, indistinguishable from those activated by metabotropic GLU receptors, can be activated by high-frequency stimulation of the TS. We also noted depression of the EPSP/IPSP complex following high-frequency stimulation of the TS. This could result from stimulation of presynaptic metabotropic receptors by synaptically released GLU and provide a physiological correlate of the presynaptic effects of 1*S*,3*R*-ACPD we have reported. However, such an interpretation would be premature for several reasons. First of all, rapid stimulation of postsynaptic 6-cyano-7-dinitroquinoxaline-2,3-dione–sensitive AMPA receptors causes them to desensitize (Kiskin et al., 1986). This would reduce the size of the EPSP and, indirectly, the size of the GLU-driven component of the polysynaptic IPSP. Second, other types of presynaptic glutamate receptors exist [e.g., the L-aminophosphonobutyrate (L-AP4) receptor] whose activation also leads to a depression of GLU release (Forsythe and Clements, 1990). Thus, without specific antagonists of the L-AP4 and metabotropic GLU receptors, the relative contributions of these two entities are difficult to assess.

Until recently, activation of metabotropic glutamate receptors has been associated with stimulation of phospholipase C and mobilization of intracellular Ca^{2+} stores (Palmer et al., 1989; Schoepp and Johnson, 1989; Schoepp et al., 1990). In some instances these effects have been shown to be blocked by L-AP3 (Miller, 1991). It is clearly important to try to relate these observations to the recently observed electrophysiological effects of 1*S*,3*R*-ACPD. As we have now demonstrated, at least some of these effects can be dissociated from any ability to mobilize Ca^{2+} from intracellular stores and are insensitive to the effects of L-AP3. This conclusion was also reached by Charpak et al. (1990), who found that although activation of metabotropic receptors sometimes resulted in Ca^{2+} mobilization in hippocampal pyramidal neurons, this effect appeared to be dissociated from the ability to depolarize the same cells.

The electrophysiological effects of 1*S*,3*R*-ACPD have been recently examined in various regions of the brain, particularly the hippocampus and the cerebellum. The present results are similar to those reported for the hippocampus where 1*S*,3*R*-ACPD excites pyramidal neurons (Stratton et al., 1989, 1990; Charpak et al., 1990) and reduces both excitatory (glutamatergic) (Baskys and Malenka, 1991; Desai and Conn, 1991; Pacelli and Kelso, 1991) and inhibitory (GABAergic) (Desai and Conn, 1991; Pacelli and Kelso, 1991) transmission. The excitatory tests of 1*S*,3*R*-ACPD in the hippocampus include direct depolarization of pyramidal neurons as well as a reduction in spike accommodation. It has been suggested that these effects are due to inhibition of $I_{K(Ca)}$ and I_m, respectively (Charpak et al., 1990). Similar mechanisms could underlie the excitatory effects of 1*S*,3*R*-ACPD in the NTS. Three types of K^+ conductances have been previously described in the NTS, these being I_m, $I_{K(Ca)}$, and I_A (Champagnat et al., 1986; Dekin and Getting, 1987). Indeed, we have observed that 1*S*,3*R*-ACPD does block I_m in these neurons (S. R. Glaum, unpublished observations). This is also consistent with the observation that the 1*S*,3*R*-ACPD–induced inward current is Ba^{2+} sensitive and TEA insensitive. However, the depolarizing effects of 1*S*,3*R*-ACPD could also involve a reduction in $I_{K(leak)}$, as recently demonstrated for thalamic neurons (von Krosigk and McCormick, 1991). The inability of 1*S*,3*R*-ACPD to mobilize Ca^{2+} in NTS neurons is not necessarily inconsistent with a possible block of I_m in these cells. Thus, although some studies have suggested that a rise in $[Ca^{2+}]_i$ leads to the inhibition of I_m (Kirkwood et al., 1991), this relationship has proved to be highly complex (Marrion et al., 1991). Other studies have suggested that activation of protein kinase C may be involved in the inhibition of I_m or indeed that IP_3 itself may alter the properties of this K^+ channel directly (Brown and Adams, 1987; Dutar and Nicoll, 1988). Thus, even in the absence of a rise in $[Ca^{2+}]_i$, one cannot rule out a role for phospholipase C activation in the signal transduction pathway activated by 1*S*,3*R*-ACPD in NTS neurons.

The molecular processes underlying the ability of 1*S*,3*R*-ACPD to reduce neurotransmitter release in the NTS and elsewhere are unclear. In some reports, activation of metabotropic glutamate receptors has been shown to block Ca^{2+} currents, which could certainly produce such an effect (Lester and Jahr, 1990; see also Sahara and Westbrook, 1991). However, this action has not always been observed (Charpak et al., 1990).

It is interesting to compare the electrophysiological effects of 1*S*,3*R*-ACPD with those previously reported for muscarinic agonists in the CNS. These include decreasing or increasing a variety of K^+ conductances, presynaptic inhibition, and inhibition of Ca^{2+} currents (reviewed in Nicoll et al., 1990). It seems likely that these effects result from stimulation of different subclasses of muscarinic receptors, at least five of which appear to exist in the CNS (Bonner, 1989). It is likely that the variety of effects produced by 1*S*,3*R*-ACPD also results from activation of a family of metabotropic GLU receptors. Indeed, although the sequence of one of these receptors has been elucidated using molecular biological techniques, several incompletely characterized isoforms clearly exist as well (Houamed et al., 1991; Ohishi et al., 1991). Heterogeneity of metabotropic GLU receptors is also suggested by considering the effects of the putative metabotropic GLU receptor antagonist L-AP3. Although many of the effects of 1*S*,3*R*-ACPD in the NTS and hippocampus are clearly resistant to block by this compound, it does seem to be effective in cultured neurons from the cerebellum (Irving et al., 1990; Linden et al., 1991), suggesting a difference in receptor specificity.

The present results support the notion that the kynurenic acid–insensitive effects of GLU on the baroreceptor reflex observed *in vivo* may be the result of metabotropic GLU receptor activation. High-frequency stimulation of afferent projections to the NTS through the TS seems to be able to produce postsynaptic excitation through the activation of metabotropic GLU receptors. It should also be pointed out that such high-frequency stimulation in the region of the TS has been shown to lead to a progressive depression in PSP amplitude *in vitro* (Miles, 1986), most likely through a presynaptic mechanism. Our results may also offer an explanation for this phenomenon. Such synaptic depression may underlie the central adaptation of cardiac responses to baroreceptor stimulation in that, by favoring inhibition of inhibitory neurons, metabotropic GLU receptor activation may enhance vagal output of the NTS during intense activation of baroreceptor afferents. While the importance of neurons inhibited by baroreceptor activation is poorly understood (Mifflin and Felder, 1990), by favorably reducing inhibitory mechanisms, vagal outflow could be increased. Finally, it is also clear that the development of further specific agents that act as metabotropic GLU receptors would not only provide useful tools for elucidating the physiological roles of such receptors, but could also provide novel therapeutic agents for the control of blood pressure and a variety of other important visceral processes.

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