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Inhibition of Phosphoprotein Phosphatases Blocks Metabotropic Glutamate Receptor Effects in the Rat Nucleus Tractus Solitarii

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SUMMARY

Whole-cell recordings were made from dorsomedial nucleus tractus solitarii neurons in thin coronal medullary slices of the rat, at the level of the area postrema. Monosynaptic excitatory postsynaptic currents (EPSCs) were evoked in the tractus solitarius by electrical stimulation in the presence of D-2-amino-5-phosphonopentanoic acid (AP5) and bicuculline. Currents were also evoked by pressure ejection of (*S*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) in the presence of AP5, bicuculline, and tetrodotoxin or muscimol in the presence of 6,7-dinitroquinoxaline-2,3-dione and AP5. The metabotropic glutamate receptor (mGluR) agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate [(1*S*,3*R*)-ACPD] reversibly depressed the EPSC and muscimol currents and reversibly potentiated AMPA currents. The effects of (1*S*,3*R*)-ACPD were blocked in the presence of a low concentration of the phosphoprotein phosphatase (PP)1 and PP2A inhibitor okadaic acid (OA) but not by a low concentration of the PP inhibitor calyculin A. The immunosuppressant agent FK506 failed to block (1*S*,3*R*)-ACPD effects on AMPA currents. However, (1*S*,3*R*)-ACPD applied in the presence of FK506 produced a reversible potentiation of muscimol currents. We previously demonstrated that the cell-permeant cGMP analog 8-Br-cGMP can mimic many of the effects of (1*S*,3*R*)-ACPD. OA antagonized the effects of 8-Br-cGMP in the present investigation. Finally, we previously demonstrated that brief tetanic stimulation results in the activation of a presynaptic mGluR autoreceptor and depression of subsequently evoked EPSCs. OA similarly blocked tetanus-induced depression of EPSCs. These findings suggest that mGluRs on tractus solitarius afferents and first-order nucleus tractus solitarii neurons may modulate glutamate release and AMPA and γ-aminobutyric acid type A receptor activity via activation of one or more PPs, such as PP2A and/or calcineurin.

mGluRs are widely distributed in the mammalian central nervous system and appear to modulate synaptic transmission in part via interactions with AC and/or PI turnover (1). The compound (1*S*,3*R*)-ACPD appears to be a specific agonist at mGluRs, although its potency relative to that of quisqualate, L-2-amino-4-phosphonobutanoic acid, or glutamate varies at each mGluR subtype. Using molecular biological methods, at least seven mGluR subtypes have been described in the literature (2–4).

The NTS receives primary sensory afferents from a variety of cardiorespiratory, gastric, and other visceral organs and functions to coordinate autonomic reflexes (5). Excitatory amino acid receptors in the NTS, including mGluRs, have been shown to be integral components of certain autonomic reflexes such as the cardiac baroreflex (6, 7). We have previously described a number of mGluR-mediated electrophysiological responses to (1*S*,3*R*)-ACPD in the NTS. These include a direct excitatory effect mediated by the reduction of an inwardly rectifying $I_{K(ACPD)}$, a postsynaptic potentiation of I_{AMPA}, a postsynaptic reduction of I_{MUSC}, and a depression of both the monosynaptic IPSC and monosynaptic EPSC evoked from the region of the TS (8–12). Because the EPSC in the NTS, recorded in the presence of the NMDA receptor antagonist AP5, is mediated by synaptically released glutamate acting upon postsynaptic AMPA/kainate receptors, the inhibitory actions of (1*S*,3*R*)-ACPD on the EPSC most likely occur presynaptically (8, 9). In a similar manner, brief tetanic stimulation in the region of the TS appears to activate a presynaptic mGluR autoreceptor, presumably through the actions of endogenous glutamate, to depress the amplitude of the subsequent EPSC (10). Alternatively, because $I_{K(ACPD)}$ and mGluR-me-

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ABBREVIATIONS: mGluR, metabotropic glutamate receptor; (1*S*,3*R*)-ACPD, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; AMPA, (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP5, D-amino-5-phosphonopentanoic acid; NMDA, *N*-methyl-D-aspartate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; aCSF, artificial cerebrospinal fluid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; TTX, tetrodotoxin; NTS, nucleus of the tractus solitarius; PP, phosphoprotein phosphatase; IPSC, inhibitory postsynaptic current; EPSC, excitatory postsynaptic current; $I_{K(ACPD)}$, inward current(s) evoked by (1*S*,3*R*)-ACPD; I_{AMPA}, inward current(s) evoked by AMPA; I_{MUSC}, outward current(s) evoked by muscimol; TS, tractus solitarius; GABA, γ-aminobutyric acid; PI, phosphatidylinositol; AC, adenylate cyclase; PTP, post-tetanic potentiation; PTD, post-tetanic depression; LTP, long term potentiation; MCPG, (*S*)-α-methyl-4-carboxyphenylglycine; OA, okadaic acid; CL-A, calyculin A; PKG, protein kinase G.

diated modulatory effects on I_{AMPA} and I_{MUSC} are resistant to the effects of TTX, they are most likely mediated via the actions of a postsynaptic mGluR. It is currently unclear whether presynaptic mGluRs contribute to the observed inhibition of monosynaptic IPSCs. Notably, mGluRs in the hippocampus also directly excite pyramidal cells (13) and interneurons (14), depress excitatory glutamatergic transmission (15), mediate a LTP-related increase in postsynaptic AMPA receptor sensitivity (16, 17), and depress inhibitory GABAergic transmission (13, 14). mGluRs may also contribute to the ability of glutamate to potentiate $GABA_A$ responses in hippocampal neurons (18). Curiously, despite the observed ability of (1*S*,3*R*)-ACPD to modulate synaptic transmission in the NTS, *in situ* hybridization studies have failed to identify significant densities of the known mGluRs in this nucleus (19–21). Furthermore, we have previously presented data suggesting that mGluRs in the NTS may be coupled not to PI turnover or effects on AC but, rather, to the activation of PKG. This conclusion comes from the observation that many of the effects of (1*S*,3*R*)-ACPD are mimicked by and nonadditive with those produced by 8-Br-cGMP (11). In addition, we have demonstrated that many of these (1*S*,3*R*)-ACPD-mediated effects are blocked by the selective heme oxygenase inhibitor zinc-protoporphyrin IX (11). The production of carbon monoxide by heme oxygenase after mGluR activation could directly activate guanylyl cyclase to increase cGMP levels (11). Notably, whereas mGluR activation in the hippocampus is a requirement for the induction of LTP (22), LTP is prevented by inhibitors of heme oxygenase like zinc-protoporphyrin IX (23).

The observation that mGluR effects in the NTS might be mediated by a PKG-dependent mechanism has led us to further investigate the mGluR signaling pathway in these neurons. A role for PKG in the activation of PPs has recently emerged (24). A variety of neuronal PPs have been identified (for review, see Ref. 25), including the serine/threonine PP1 and PP2A and the Ca^{2+}/calmodulin-dependent PP calcineurin. It is also clear that AMPA and $GABA_A$ receptors can be modulated by alterations of their intracellular phosphorylation (26–30). We therefore examined whether (1*S*,3*R*)-ACPD effects in the NTS might be mediated by the activation of one or more PPs.

In the present study we used three PP inhibitors, OA, CL-A, and the immunosuppressant drug FK506 (31). FK506 can inhibit the PP calcineurin via an interaction with FK-binding proteins (31). Both OA and CL-A are inhibitors of PP1 and PP2A. In some systems, CL-A appears to be 10–100-fold more potent than OA as an inhibitor of PP1 (32–35). Although the relative inhibitory potencies of CL-A and OA for PP1 have not been compared in a brain slice preparation, we attempted to exploit the previously reported potency difference in the present study, in a preliminary effort to discriminate between PP1 and PP2A involvement in mGluR effects. We examined the effects of (1*S*,3*R*)-ACPD and the PP inhibitors on I_{AMPA} and I_{MUSC}. We also examined the effects of PP inhibitors on the modulation of EPSCs by endogenously released glutamate acting on the presynaptic mGluR autoreceptor (10).

Materials and Methods

Experiments were performed on 67 neurons in separate coronal brainstem slices prepared from Holtzman rats of age 18–36 days, as described previously (8–10). Briefly, rats were given overdoses of ether and 175–225-μm slices of the medulla were prepared with a vibrating microtome. After a recovery period (≥1 hr) at 33°, a single slice was transferred to the recording chamber and continuously perfused (4–6 ml/min) with aCSF (containing the following, in mM: NaCl, 126; $NaHCO_3$, 26.2; NaH_2PO_4, 1; KCl, 3; $MgSO_4$, 1.5; $CaCl_2$, 2.5; glucose, 10) at 25° and continuously gassed with 95% O_2/5% CO_2.

All experiments were performed under voltage-clamp conditions, with currents being recorded under discontinuous single-electrode voltage clamp (V_{hold}, −50 to −70 mV; 7–14 kHz) with whole-cell patch electrodes containing the following (in mM): potassium gluconate, 145; $MgCl_2$, 2; $CaCl_2$, 0.1; HEPES, 5; EGTA, 1.1; K_2ATP, 5; pH 7.2). Estimates of the series resistance, made in the bridge mode of the amplifier, were between 10 and 20 MΩ (8–10). A bipolar stimulating electrode was placed in the ipsilateral TS and recordings were made from neurons dorsomedial to the TS and adjacent to the area postrema. This region of the NTS contains a high density of cardiorespiratory neurons receiving baroreceptor afferent projections (6). Synaptic currents were pharmacologically isolated as described previously (8). Briefly, EPSCs were evoked (0.1 Hz, 4–11 V, 300 μsec) in the presence of AP5 (50 μM) and the $GABA_A$ antagonist bicuculline (10 μM). Tetanizing stimuli (20 Hz, 2 sec) were delivered at the control intensity. I_{AMPA} were evoked by micropressure ejection (15 msec, 2–10 psi) of (*S*)-AMPA (10 mM), in bicuculline/AP5-containing aCSF supplemented with 1 μM TTX, from a blunt patch pipette directed at the soma under visual guidance. I_{MUSC} were similarly evoked by micropressure ejection of muscimol (10 mM) in AP5/DNQX/TTX-supplemented aCSF. (1*S*,3*R*)-ACPD (20 μM), 8-Br-cGMP (100 μM), (*S*)-MCPG (100 μM), CL-A (2–100 nM), OA (2 or 20 nM), *l*-norokadaone (20 nM), and FK506 (100 nM) were delivered in the perfusate.

Agonists [(1*S*,3*R*)-ACPD or 8-Br-cGMP] were applied in the perfusate for 45–70 sec. MCPG or one of the PP inhibitors was applied in the perfusate for >5 min (MCPG) or >7 min (other compounds) before agonist coapplication. The mean agonist percentage of control current [mean (n = 3–5) peak current in the presence of agonist/mean (n = 3) peak current immediately before agonist application) × 100] was compared with the values recorded in the presence of MCPG or a PP inhibitor. Values recorded in the presence of MCPG or one of the PP inhibitors alone were not significantly different from those obtained in aCSF alone. For experiments examining post-tetanic responses, the mean peak current of the three EPSCs evoked immediately after a single 20-Hz, 2-sec tetanus was compared with the mean peak control current of the three EPSCs immediately before the tetanus and was expressed as a percentage. In some instances, more than one inhibitor was applied and a single tetanus was delivered at 7–10-min intervals, a paradigm that results in no decay of the control response in the absence of antagonists (10). Values were determined as before and pooled according to the initial response to tetanus (i.e., PTP or PTD). Values were reported as mean ± standard error. Significant differences ($p < 0.05$) were determined by one-way analysis of variance for repeated measures followed by Student-Newman-Keuls test, with the exception of experiments examining tetanus-induced modulation of the EPSC, where *post hoc* comparisons were made by Bonferroni t test. In separate experiments examining the effects of 100 nM CL-A on I_{AMPA}, comparisons were made by paired t test.

Results

Activation of mGluR by (1*S*,3*R*)-ACPD results in a reversible postsynaptic potentiation of I_{AMPA} in rat Purkinje (36), dorsal horn (37), and NTS (9) neurons. In the present investigation, 20 μM (1*S*,3*R*)-ACPD increased I_{AMPA} to 157.0 ± 11.2% of control ($p < 0.05$, n = 15), as illustrated in Fig. 1A. The size of I_{AMPA} was not affected by addition of CL-A (2 nM) to the bath for 7–10 min. In addition, (1*S*,3*R*)-ACPD induced a similar increase in I_{AMPA} in the presence of 2 nM CL-A (146.6 ± 2.8% of control, not significant, n = 3). However, at a concentration of 100 nM, CL-A did reduce the potentiation of I_{AMPA} by (1*S*,3*R*)-ACPD (112.4 ± 14.6% of control, $p < 0.05$, n = 3). Fig. 1B illustrates that the control I_{AMPA} and potentiation by

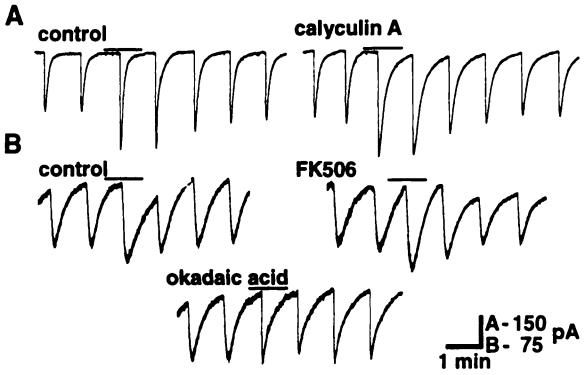

Fig. 1. Selective inhibition of PP2A blocks potentiation of I_{AMPA} by (1*S*,3*R*)-ACPD in the dorsomedial NTS. A, Continuous chart record of inward current responses to pressure-applied (*S*)-AMPA (*downward deflections*), illustrating reversible potentiation in the presence of (1*S*,3*R*)-ACPD (20 μM) (*bar*). (1*S*,3*R*)-ACPD induces a similar potentiation of I_{AMPA} in the presence of 2 nM CL-A. CL-A was applied in the perfusate for 8 min before and throughout the *trace*. I_{AMPA} were evoked from a V_{hold} of −60 mV in the presence of TTX (1 μM), AP5 (50 μM), and bicuculline (10 μM). B, Control I_{AMPA} potentiation by (1*S*,3*R*)-ACPD in a second dorsomedial NTS neuron, recorded as in A. After a 9-min preincubation with the calcineurin antagonist FK506 (100 nM), I_{AMPA} were similarly potentiated. The slice was washed for 4 min before a 7-min preincubation with OA (20 nM). No potentiation of I_{AMPA} by (1*S*,3*R*)-ACPD was noted in the presence of OA.

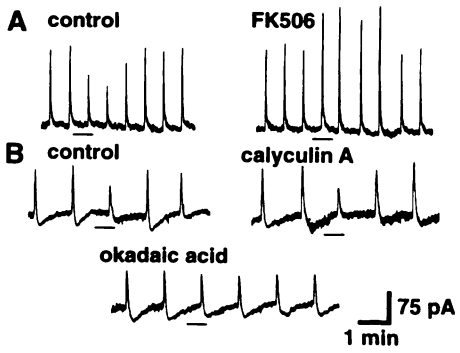

Fig. 2. OA and FK506 differentially modulate (1*S*,3*R*)-ACPD effects on I_{MUSC}. A, Continuous chart record of outward current responses to pressure-applied muscimol (*upward deflections*) in the dorsomedial NTS, illustrating the reversible reduction observed in the presence of (1*S*,3*R*)-ACPD (20 μM) (*bar*). In contrast, (1*S*,3*R*)-ACPD induces potentiation of I_{MUSC} in the presence of 100 nM FK506. FK506 was applied in the perfusate for 8 min before and throughout the *trace*. I_{MUSC} were evoked from a V_{hold} of −50 mV in the presence of AP5 (50 μM) and DNQX (10 μM). B, Control I_{MUSC} reduction by (1*S*,3*R*)-ACPD in a second dorsomedial NTS neuron, recorded as in A. After a 7-min preincubation with the PP1 inhibitor CL-A (2 nM), I_{MUSC} were similarly reduced. The slice was washed for 6 min before an 8-min preincubation with OA (20 nM). No reduction of I_{MUSC} by (1*S*,3*R*)-ACPD was noted in the presence of OA.

(1*S*,3*R*)-ACPD produced in another cell were unaffected by the calcineurin antagonist FK506 (179.9 ± 27.1% of control, not significant, $n = 5$). In contrast, exposing the slice to OA (20 nM) for 7–10 min significantly reduced the (1*S*,3*R*)-ACPD-mediated potentiation of I_{AMPA} (89.0 ± 7.8% of control, $p < 0.05$, $n = 10$) without affecting control responses. Because these data suggested that OA was not only blocking but also perhaps revealing an underlying 'reversal' of the effect of (1*S*,3*R*)-ACPD on I_{AMPA} (i.e., reduction rather than potentiation), a 100 nM concentration of OA was evaluated in three test cells. Although similar antagonist actions of OA were observed, we failed to note a significant (1*S*,3*R*)-ACPD-mediated reduction of I_{AMPA} in the presence of 100 nM OA (91.3 ± 6.5% of control, not significant, $n = 3$). The ability of 20 nM OA to block (1*S*,3*R*)-ACPD effects on I_{AMPA} was reversible after extensive (10–40-min) washout, where examined (data not shown).

A majority of dorsomedial NTS neurons appear to receive an inhibitory GABAergic input from interneurons located in and around the TS (38). We previously demonstrated that (1*S*,3*R*)-ACPD reversibly reduced both the monosynaptic $GABA_A$-mediated IPSC and I_{MUSC} in these neurons (8, 9). As illustrated in Fig. 2A, (1*S*,3*R*)-ACPD depressed I_{MUSC} in the present study (40.7 ± 4.9% of control, $p < 0.05$, $n = 16$). In contrast, I_{MUSC} was significantly potentiated by (1*S*,3*R*)-ACPD in the presence of FK506 (138.8 ± 19.9% of control, $p < 0.05$, $n = 6$). As before, control I_{MUSC} was unchanged by FK506 alone. This reversal of (1*S*,3*R*)-ACPD effects on I_{MUSC} appeared to be specific to FK506, as shown in Fig. 2B. Thus, although CL-A failed to block (1*S*,3*R*)-ACPD effects (56.1 ± 14.2% of control, not significant, $n = 4$), whereas 20 nM OA again proved to be an effective antagonist (101.6 ± 1.7% of control, $p < 0.05$, $n = 9$), neither resulted in a significant potentiation of I_{MUSC}, alone or in combination with (1*S*,3*R*)-ACPD.

We previously reported that presynaptic mGluR, activated either by exogenous (1*S*,3*R*)-ACPD (8) or by endogenous glutamate released immediately afte a brief tetanic stimulus (10), inhibited the EPSC evoked from the region of the TS. In the present study, we examined whether 2 nM and 20 nM OA could inhibit the ability of (1*S*,3*R*)-ACPD to reduce the EPSC. Under control conditions, 20 μM (1*S*,3*R*)-ACPD reduced the EPSC evoked by 0.1-Hz stimulation to 28.9 ± 5.6% of control ($p < 0.05$, $n = 16$). In the presence of 2 nM OA, the effect of (1*S*,3*R*)-ACPD was significantly attenuated [61.9 ± 5.9% of control, $p < 0.05$ versus (1*S*,3*R*)-ACPD alone, $n = 3$]. OA at 20 nM further reduced (1*S*,3*R*)-ACPD-mediated inhibition of the EPSC [95.9 ± 2.7% of control, $p < 0.05$ versus (1*S*,3*R*)-ACPD alone, $n = 4$]. Compared with OA, CL-A proved to be a less efficacious, although in all cases a significant, antagonist of the effects of (1*S*,3*R*)-ACPD on the EPSC. In the presence of CL-A at 2, 20, and 100 nM, (1*S*,3*R*)-ACPD reduced the EPSC to 54.8 ± 4.2, 52.7 ± 4.9, and 67.6 ± 6.3% of control, respectively [all $p < 0.05$ versus (1*S*,3*R*)-ACPD alone, $n = 4$ for all concentrations]. Finally, the inactive OA analog *l*-norokadaone (20 nM) failed to significantly inhibit (1*S*,3*R*)-ACPD-mediated inhibition of the EPSC [28.9 ± 9.4% of control, not significant versus (1*S*,3*R*)-ACPD alone, $n = 4$].

We further examined the effects of PP inhibitors on the presynaptic autoreceptor mediating (1*S*,3*R*)-ACPD inhibition of the EPSC. As we reported previously, in the majority of recordings made in the dorsomedial NTS a 20-Hz, 2-sec tetanus delivered in the presence of NMDA and $GABA_A$ receptor antagonists resulted in a subsequent PTD of the monosynaptic, DNQX-sensitive EPSC (10). In the presence of the selective mGluR antagonist MCPG (39), all cells displayed PTP, suggesting that a presynaptic inhibitory mGluR autoreceptor is activated by endogenous glutamate in this slice preparation (10, 12). As shown in Fig. 3A, OA, but not CL-A or FK506, mimicked the effects of MCPG and produced PTP in cells previously showing PTD. In the remaining recordings, PTP was evident under control conditions (Fig. 3B; see also Ref. 10). In these neurons, MCPG produced a significant enhancement of PTP. As shown in Fig. 3B, OA, but not CL-A or FK506, mimicked the effects of MCPG and produced enhanced PTP in this subpopulation of NTS neurons. The results of the experiments described above are summarized in Table 1.

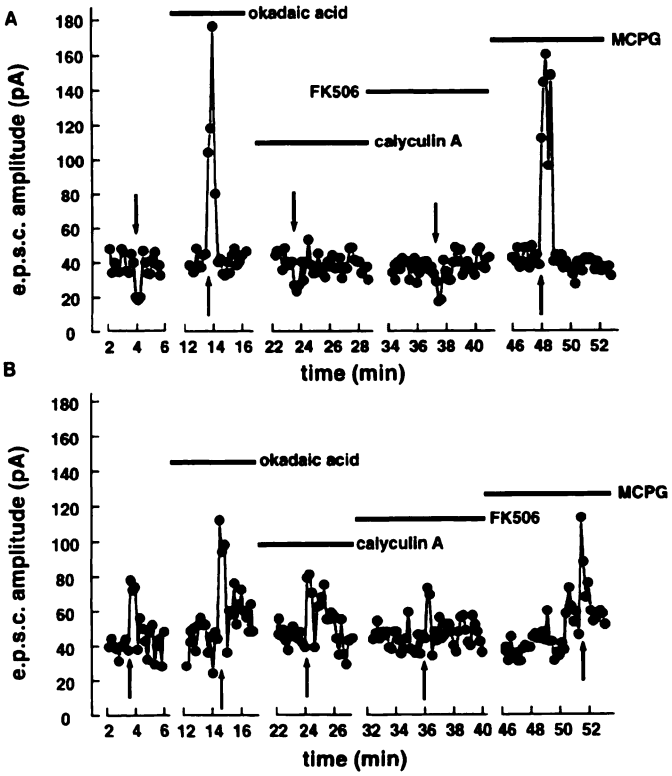

Fig. 3. Dot plot of peak EPSC amplitude in an NTS neuron, illustrating PP inhibitor effects on tetanus-induced PTD and PTP. A, The EPSC evoked by electrical stimulation in the ipsilateral TS (0.1 Hz) undergoes PTD after a 20-Hz, 2-sec tetanus (*arrows*). Similar PTD is observed in the majority of recordings (see Table 1). PTP is observed when the tetanus (*arrows*) is applied in the presence of OA (20 nM) but not CL-A (2 nM) or FK506 (100 nM). The mGluR antagonist MCPG (100 μM) similarly converts PTD to PTP. The EPSC was recorded from a V_{hold} of −60 mV in the presence of AP5 (50 μM) and bicuculline (10 μM). B, In an NTS neuron exhibiting PTP after a 20-Hz, 2-sec tetanus (*arrows*), PTP is enhanced in the presence of OA and MCPG but not CL-A or FK506. Recording conditions and drug concentrations were as in A.

TABLE 1

Effects of PP inhibitors and the selective mGluR antagonist MCPG on tetanus-induced PTD and PTP of the monosynaptic EPSC in dorsomedial NTS neurons

Cells were grouped by response of the monosynaptic EPSC to a 20-Hz, 2-sec tetanus, as described in Materials and Methods. OA (20 nM), CL-A (2 nM), FK506 (100 nM) or MCPG (100 μM) was delivered in the perfusate for 7–10 min before tetanic stimulation was repeated. Significance versus control was determined by one-way analysis of variance for repeated measures and Bonferroni *t* test. Values are mean ± standard error.

	Treatment	EPSC (% control)	$p < 0.05$ versus control	*n*
		pA		
PTD (control)	None	79.8 ± 4.3		14
	OA	128.8 ± 13.8	Yes	6
	CL-A	68.5 ± 7.4	No	4
	FK506	81.4 ± 5.2	No	3
	MCPG	130.0 ± 7.4	Yes	9
PTP (control)	None	134.2 ± 15.2		9
	OA	195.6 ± 17.8	Yes	6
	CL-A	132.4 ± 18.8	No	4
	FK506	133.8 ± 27.0	No	4
	MCPG	234.8 ± 35.2	Yes	9

The aforementioned results suggested that PP inhibitors interfere with mGluR-mediated modulation of glutamate release and AMPA and $GABA_A$ receptor function. However, an alternative interpretation could be that these compounds altered the activity of the mGluR itself (e.g., desensitization), rather than acting further along the signal transduction pathway. To examine this possibility, we used the cell-permeant cGMP analog 8-Br-cGMP, which we previously demonstrated mimicked the effects of (1*S*,3*R*)-ACPD on the EPSC, I_{AMPA}, and I_{MUSC} (11). As illustrated in Fig. 4A, the EPSC was unaffected by 8-Br-cGMP in the presence of OA (92.8 ± 7.7% of control, not significant, $n = 4$). However, after extensive (10–20-min) washout of OA, 8-Br-cGMP significantly reduced the EPSC (29.4 ± 13.6% of control, $p < 0.05$, $n = 4$), as shown in Fig. 4B. Similarly, 8-Br-cGMP was ineffective at potentiating I_{AMPA} in the presence of OA (99.5 ± 5.0% of control, not significant, $n = 3$) but was fully effective after OA washout (243.4 ± 31.7% of control, $p < 0.05$, $n = 3$), as illustrated in Fig. 5A. Finally, Fig. 5B demonstrates that 20 nM OA similarly blocked the 8-Br-cGMP-mediated reduction in I_{MUSC} (94.5 ± 4.4% of control in OA, not significant; 39.9 ± 10.7% of control after 20 nM OA washout, $p < 0.05$, $n = 4$). We have previously shown that the effects of a supramaximal concentration of (1*S*,3*R*)-ACPD on the aforementioned parameters are virtually identical to and completely nonadditive with those of 8-Br-cGMP (11). Similarly, no qualitative differences were noted between the responses to (1*S*,3*R*)-ACPD or 8-Br-cGMP in the presence of OA, suggesting that they may target similar effector systems.

Discussion

A variety of second messenger systems have been described for mGluRs in central nervous system neurons. mGluRs 1 and 5 appear to favor activation of PI turnover in the systems examined, whereas mGluRs 2, 3, 4, 6, and 7 may have differential effects on AC (1–3, 40, 41). We have previously provided evidence that mGluRs may also be coupled to guanylyl cyclase (10), perhaps through the liberation of a gaseous messenger

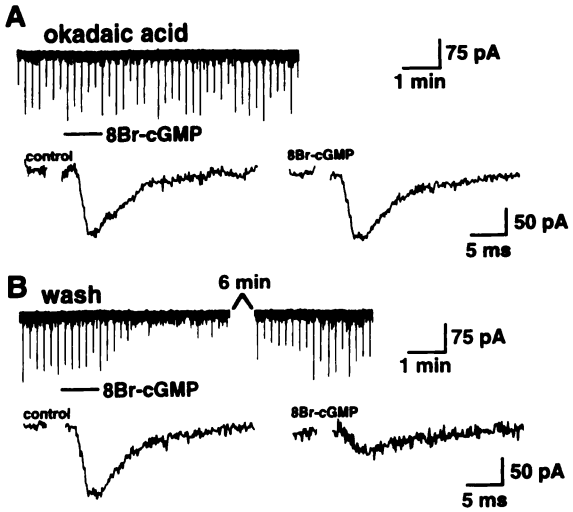

Fig. 4. Depression of evoked EPSCs by 8-Br-cGMP is blocked in the presence of OA. A, Continuous chart record of the EPSC (*downward deflections*) evoked by 0.1-Hz electrical stimulation in the ipsilateral TS in the presence of AP5 (50 μM) and bicuculline (10 μM). High-resolution *traces* are averages of three consecutive EPSCs taken immediately before (*control*) and 2 min after (*8Br-cGMP*) the onset of bath application of 100 μM 8-Br-cGMP (*bar*). OA was continuously applied in the perfusate for 7 min before and throughout the *trace* shown. The V_{hold} was −55 mV. B, Evidence that 8-Br-cGMP (100 μM) (*bar*) rapidly and reversibly inhibits the EPSC in the same cell as A after a 12-min washout of OA. High-resolution *traces* and recording conditions were as in A.

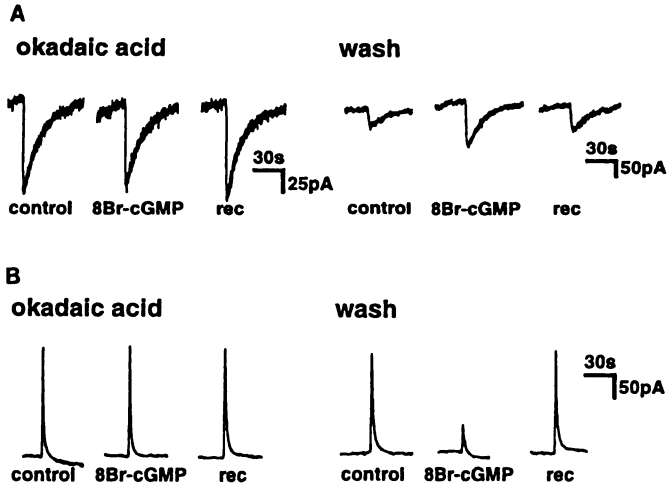

Fig. 5. OA reversibly inhibits the effects of 8-Br-cGMP to potentiate AMPA and reduce I_{MUSC} in the NTS. A, The inward current responses to pressure application of (*S*)-AMPA (*downward deflections*) are not potentiated by 8-Br-cGMP (100 μM) in the presence of OA (20 nM). OA was applied in the perfusate for 8 min before the control recording and throughout the subsequent records. The 8-Br-cGMP record was taken 2 min after the onset of a 1-min bath application of 8-Br-cGMP. The recovery record (*rec*) was recorded 10 min after the application of 8-Br-cGMP. Recordings were made in the presence of AP5 (50 μM) and bicuculline (10 μM) from a V_{hold} of −60 mV. After a 15-min washout of OA (*wash*), 8-Br-cGMP reversibly potentiated I_{AMPA}. *Trace* recordings and 8-Br-cGMP applications were as described previously. B, The outward current responses to pressure-applied muscimol (*upward deflections*) are not potentiated by 8-Br-cGMP in the presence of OA. I_{MUSC} were evoked in the presence of AP5 (50 μM) and DNQX (10 μM) at a V_{hold} of −50 mV. Application of drugs and *trace* recordings were otherwise as described in A, where 8-Br-cGMP effects were noted after washout of OA.

such as carbon monoxide (11). The events occurring between the presumed mGluR-mediated increase in intracellular cGMP and the observed modulatory effects on AMPA and $GABA_A$ receptors are not clear. Two likely routes for cGMP-dependent signaling are activation of PKG and activation of phosphodiesterase, which could lower intracellular cAMP levels (42). If the latter were true, then increasing intracellular cAMP levels would be expected to block or reverse the effects of (1*S*,3*R*)-ACPD. However, we previously found few effects of cell-permeant cAMP analogs in the NTS (9). In contrast, cGMP appears to activate potassium channels via dephosphorylation secondary to PKG-mediated activation of an OA-sensitive PP in rat pituitary tumor cells (24). Similarly, the results of the present investigation suggest that dephosphorylation events, mediated by one or more PPs, may be involved in mGluR effects in the NTS. At present, none of the PP inhibitors has been sufficiently characterized in a neuronal system for unequivocal determination of the involvement of a particular PP isoform. In particular, neither OA nor CL-A may be considered selective between PP2A and PP1, although CL-A appears to be up to 100-fold more potent than OA in inhibiting PP1 in certain other systems (32–35, 43). Thus, although we cannot unambiguously determine which PP may be involved in the observed mGluR-mediated effects in the NTS, the finding that CL-A was a less potent antagonist of (1*S*,3*R*)-ACPD effects than was OA, where tested, may indicate the preferential involvement of PP2A in the present results. In addition, the unexpected reversal of (1*S*,3*R*)-ACPD modulation of I_{MUSC} observed in the presence of FK506 could indicate an involvement of calcineurin in the modulation of $GABA_A$ receptors in the NTS. It is interesting to hypothesize that mGluR may interact with other neuromodulatory mechanisms *in vivo* to reveal this otherwise 'masked' response to mGluR activation.

Modulation of ionotropic amino acid receptors, particularly AMPA/kainate, NMDA, and $GABA_A$ receptors, by protein kinases has been widely observed (16, 17, 27, 44–46). However, less is known about the role of PPs in ionotropic receptor modulation (30). In radioligand binding experiments, $GABA_A$ receptor binding is increased in adult rat neocortex after dephosphorylation (28, 29), although it is unclear what effects this may have on receptor function. More telling, 'rundown' of $GABA_A$ currents in acutely dissociated pyramidal neurons is suppressed when a supply of high-energy phosphates (e.g., Mg^{2+}/ATP) is made available in whole-cell recordings (26). This finding is compatible with the notion that mGluR activation of a PP could lead to a decrease in $GABA_A$ current, as proposed by the results of the present study. Clearly though, the finding that (1*S*,3*R*)-ACPD can positively modulate $GABA_A$ receptor function in the presence of FK506 could indicate that the regulation of these receptors by phosphorylation/dephosphorylation is substantially more complex (47).

The role of protein kinases and PPs in excitatory amino acid receptor regulation is also likely to involve multiple interrelated components. For example, in chick Purkinje neurons activation of mGluRs results in a suppression of I_{AMPA} (48). Although the direction of AMPA receptor regulation in these cells is opposite to that observed in the NTS, both appear to be cGMP-dependent phenomena (48). Whether the mGluRs in the chick Purkinje cells regulate AMPA receptors via an interaction with PPs is unknown. Notably, Lieberman and Mody (49) have reported that NMDA receptors in acutely isolated adult rat hippocampal neurons undergo differential modulation via the activation of multiple PPs, with increases in channel opening being observed in the presence of OA and decreases being observed in the presence of FK506. Similarly, AMPA/kainate receptors may also be regulated by PPs, inasmuch as a Cl^--dependent increase in AMPA receptor ligand binding in rat neocortex was recently shown to be inhibited by PP inhibitors (28). As was the case for the putative PP modulation of $GABA_A$ receptor binding described above, the functional implications of this latter study are unclear at present.

It is increasingly evident that ionotropic amino acid receptors may be both positively and negatively modulated by the selective removal of phosphates from one or more intracellular binding domains. PPs may also indirectly affect receptor phosphorylation states via a negative interaction with protein kinases, thus inhibiting further phosphorylation. In addition, guanylyl cyclases are themselves subject to regulation by phosphorylation/dephosphorylation (50). Thus, it will be important to examine at the single-channel level the regulation of AMPA and $GABA_A$ receptor function in response to PP activation. It is likely that the phosphorylation state of ionotropic amino acid receptors is an important short (and potentially long) term modulator of receptor activity. Finally, because the dorsomedial NTS is richly endowed with GABAergic interneurons (38) and mGluR activation potently inhibits monosynaptically evoked IPSCs (8), it may now be possible to examine whether a presynaptic mGluR exists on these interneurons.

Note Added in Proof

We recently reported that mGluR on NTS interneurons reduce synaptic transmission. (Glaum, S R., S. Yi, and R. J. Miller. Inhibition of protein kinase G (PKG) blocks post-, but not pre-synaptic metabotropic glutamate receptor

(mGluR) effects on inhibitory transmission in the rat nucleus tractus solitarii (NTS). *J. Physiol. (Lond.)*, in press).

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