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European Journal of Pharmacology 353 (1998) 43–51

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Type I and II metabotropic glutamate receptors regulate the outflow of [^{3}H]D-aspartate and [^{14}C]γ-aminobutyric acid in rat solitary nucleus

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Received 22 January 1998; revised 7 May 1998; accepted 15 May 1998

Abstract

Metabotropic glutamate (mGlu) receptors modulating amino acid outflow were examined in a model system in order to further characterize the pharmacological nature of the mGlu receptors involved in viscerosensory processing in the nucleus tractus solitarii. The actions of a number of subtype-selective mGlu receptor agonists and antagonists were monitored on the K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]γ-aminobutyric acid (GABA) from superfused slices of rat nucleus tractus solitarii. (±)1*S*,3*R*-1-Amino-cyclopentane-1,3-dicarboxylate (10–300 μM), produced a concentration-dependent increase in outflow, which was attenuated by a number of phenylglycine antagonists. (2*S*,3*S*,4*S*)-α-(Carboxycyclopropyl)-glycine (30–300 μM) had mixed effects on outflow. The type I-selective agonist (*RS*)-3,5-dihydroxyphenylglycine (300 μM) also increased outflow and these effects were reversed by the type I antagonist (*RS*)-1-aminoindan-1,5-dicarboxylate (100 μM). Activation of type II mGlu receptors with (2*R*,4*R*)-aminopyrrolidine-2,4-dicarboxylate (300 μM), however, decreased outflow, and this effect was antagonized by the type II antagonist LY307452 (200 μM). Interestingly, LY307452 (200 μM) alone, enhanced outflow of [^{3}H]D-aspartate, but not [^{14}C]GABA. Type III mGlu receptors may not be involved in outflow of [^{3}H]D-aspartate and [^{14}C]GABA in the nucleus tractus solitarii, as L-2-amino-4-phosphonobutyrate (30–300 μM) had no effect under the present experimental conditions. These in vitro studies provide new evidence for roles for Type I and II mGlu receptors in viscerosensory processing in nucleus tractus solitarii.

Keywords: Nucleus tractus solitarii; [^{3}H]D-Aspartate; [^{14}C]GABA ([^{14}C]γ-aminobutyric acid); Outflow, in vitro; Metabotropic glutamate receptor; Superfusion

1. Introduction

The major excitatory amino acid in the mammalian central nervous system, L-glutamate (Glu), has been well-documented as having many roles in brain function, including playing an integral role in central regulation of blood pressure. Glu is a transmitter at baroreceptor afferent neurones, which have their cell bodies in the nodose ganglion and synaptic terminals in the nucleus tractus solitarii, and which convey cardiovascular information from the heart to the brain (Chalmers et al., 1992; Lawrence and Jarrott, 1996). In addition to containing local circuit glutamatergic neurons, the nucleus tractus solitarii receives a network of diverse afferent neurones utilizing excitatory amino acids (Beart et al., 1994). The two major types of Glu receptors are the ionotropic receptors, which are linked to ion-gated receptor channels and are named after their prototypic selective agonists α-amino-3-hydroxy-5-methyl-4-isoxazole propionate, *N*-methyl-D-aspartate (NMDA) and kainate (Sommer and Seeburg, 1992). Ionotropic Glu receptors, in particular the NMDA subtype, have been well-documented as having roles in cardiovascular regulation (Lawrence and Jarrott, 1996; Gieroba and Blessing, 1993).

Additionally, there is a family of metabotropic Glu (mGlu) receptors which are G-protein coupled receptors linked to multiple second messenger systems, including phosphoinositide hydrolysis, calcium mobilization and cyclic AMP accumulation (Schoepp, 1994; Pin and Duvoisin, 1995; Conn and Pin, 1997). Molecular cloning studies have provided evidence for the existence of at least eight subtypes of mGlu receptors with a number of spliced variants also having been identified. mGlu receptors can be pharmacologically distinguished from ionotropic Glu receptors by the use of selective mGlu receptor agonists,

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0014-2999/98/$19.00

including (±)1*S*,3*R*-1-amino-cyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) and L-2-amino-4-phosphonobutyrate (L-AP4) (Pin and Duvoisin, 1995; Conn and Pin, 1997). Type I mGlu receptors ($mGlu_1$ and $mGlu_5$ receptors) are linked to the stimulation of phosphoinositide hydrolysis, while Type II ($mGlu_2$ and $mGlu_3$ receptors) and Type III mGlu receptors ($mGlu_{4,6,7 \text{ and } 8}$ receptors) are both negatively coupled to adenylate cyclase, though only Type III mGlu receptors are L-AP4-sensitive. A possible role for mGlu receptors in cardiovascular regulation has also recently been reported in a number of brainstem areas including nucleus tractus solitarii. 1*S*,3*R*-ACPD, when microinjected into nucleus tractus solitarii of anaesthetized rats produces depressor and bradycardic responses (Pawloski-Dahm and Gordon, 1992). Other electrophysiological studies in coronal medullary slices containing nucleus tractus solitarii have demonstrated that 1*S*,3*R*-ACPD can depolarize nucleus tractus solitarii neurones and activate postsynaptic mGlu receptors (Glaum and Miller, 1992, 1993), and that these responses can be antagonized by a number of mGlu receptor phenylglycine antagonists (Glaum et al., 1994). In addition, roles for mGlu receptors in respiration have been described in urethane anaesthetized rats where 1*S*,3*R*-ACPD, when microinjected into the nucleus tractus solitarii, inhibits respiratory activity and produces apnea and hypertension (Vitagliano et al., 1995).

Pre-synaptic mGlu receptors regulating the release of excitatory amino acids have been studied in a number of model systems including in vitro in cerebrocortical (Herrero et al., 1994) and striatal synaptosomes (East et al., 1995), cortical and striatal slices (Lombardi et al., 1994, 1996) and in vivo in striatum (Arai et al., 1996), nucleus accumbens (Taber and Fibiger, 1995) and nucleus tractus solitarii (Jones et al., 1998). Recent evidence suggests that depending upon the subtype of mGlu receptor to be activated, there will be either an enhancement or inhibition of

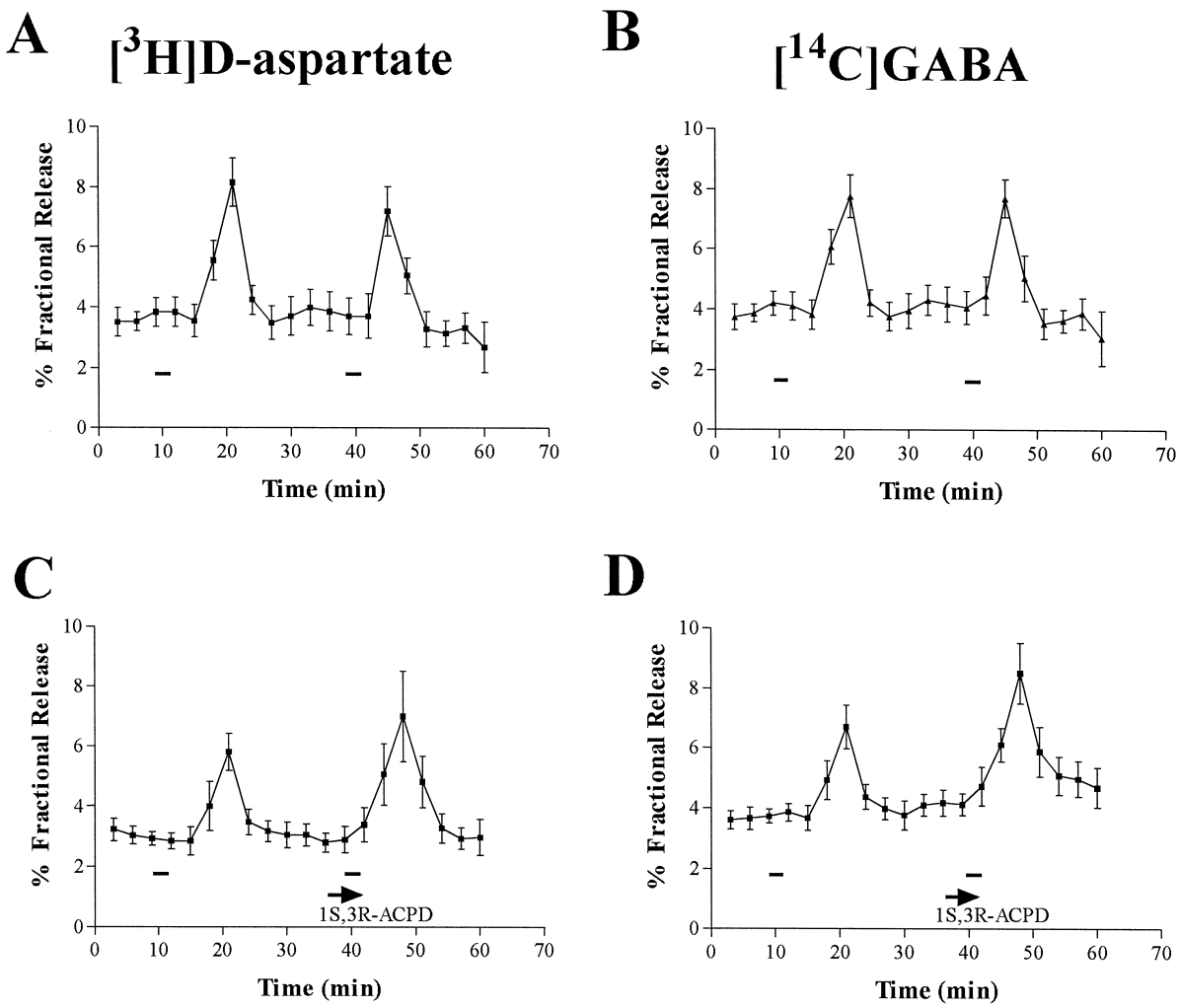


Fig. 1. Effect of high K^+ (30 mM) stimulation with and without 1*S*,3*R*-ACPD (30 μM) on [^{3}H]D-aspartate and [^{14}C]GABA outflow from slices of rat nucleus tractus solitarii. Slices were exposed to two periods (2 min) of high K^+ (bars, S_1 and S_2) in the absence or presence of tACPD (30 μM). Data are given as percentage fractional release and are the mean ± S.E.M. of 6–24 observations. Two periods of high K^+-stimulation produced reproducible increases in outflow of [^{3}H]D-aspartate (A) and [^{14}C]GABA (B). When 1*S*,3*R*-ACPD was included in S_2, it enhanced K^+-evoked outflow of [^{3}H]D-aspartate (C) and [^{14}C]GABA (D).

release—stimulation of release is due to activation of Type I, phosphoinositide-linked mGlu receptors, while attenuation of release may be due to adenylate cyclase-linked, Type II mGlu receptors (Lombardi et al., 1994, 1996).

Recent studies from our laboratory have shown in vivo evidence consistent with presynaptic mGlu receptors regulating amino acid release in rat nucleus tractus solitarii (Jones et al., 1998). These data support the ex vivo electrophysiological data of Glaum and Miller (1992), obtained in slices of nucleus tractus solitarii, who also reported that mGlu receptors apparently regulate the activity of both glutamatergic and GABAergic neurones (Glaum and Miller, 1993). GABAergic neurones of the nucleus tractus solitarii, like the key role implicated for Glu, appear as second order barosensitive neurones, to play an important role in viscerosensory regulation in this key cardiovascular nucleus (Beart et al., 1994; Lawrence and Jarrott, 1996). Given the involvement of mGlu receptors in regulating the release of neurotransmitters (Schoepp, 1994), this study has investigated roles for subtypes of mGlu receptors in the in vitro outflow of [^{14}C]GABA and [^{3}H]D-aspartate in slices of nucleus tractus solitarii using newly developed subtype-selective agonists and antagonists to delineate the pharmacological characteristics.

2. Materials and methods

2.1. Materials

(±)1*S*,3*R*-ACPD, L-AP4, (±)-α-methyl-4-carboxyphenylglycine (MCPG), (*S*)-4-carboxyphenylglycine (4CPG), (*S*)-4-carboxy-3-hydroxyphenylglycine (4C3HPG), (*RS*)-3,5-dihydroxyphenylglycine (DHPG), (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)-glycine (CCG-I) and (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA) were obtained from Tocris Cookson, Bristol, UK. (2*S*,4*S*)-2-Amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-doic acid (LY307452) and (2*R*,4*R*)-aminopyrrolidine-2,4-dicarboxylate (APDC) were synthesized at the Lilly Research Laboratories (Indianapolis, IN, USA). Where appropriate, 1.1 equivalents of NaOH was employed to aid solubilization followed by further dilutions in KH buffer. NO-711 hydrochloride was purchased from Research Biochemicals International (Natick, MA, USA). β-alanine was a gift from Professor G. Johnston (Sydney, Australia) and γ-vinyl GABA (D,L-4-aminohex-5-enoic acid) was a gift from Dr. N. Seiler, Marion Merrell Dow Research Institute (Strasbourg, France). D-[2,3-^{3}H]aspartatic acid (specific activity, 11.5 Ci/mmol) and γ-[^{14}C(U)]aminobutyric acid (specific activity, 223 mCi/mmol) were purchased from NEN Research Products (Wilmington, DE, USA). All other chemicals were of analytical grade and from various commercial suppliers.

2.2. Superfusion experiments

Experiments were performed in accordance with the National Health and Medical Research Council (NH and MRC) Code of practice for the care and use of animals for experimental purposes in Australia. Male Wistar–Kyoto rats (250–350 g) were deeply anaesthetized with nembutal and underwent transcardiac perfusion with ice-cold Krebs Henseleit (KH) buffer containing 118-mM NaCl, 4.7-mM KCl, 25-mM $NaHCO_3$, 1.2-mM KH_2PO_4, 2.3-mM $CaCl_2$, 1.2-mM $MgSO_4$ and 11-mM D(+)-glucose. Subsequently, the brain was removed and the brainstem was dissected out

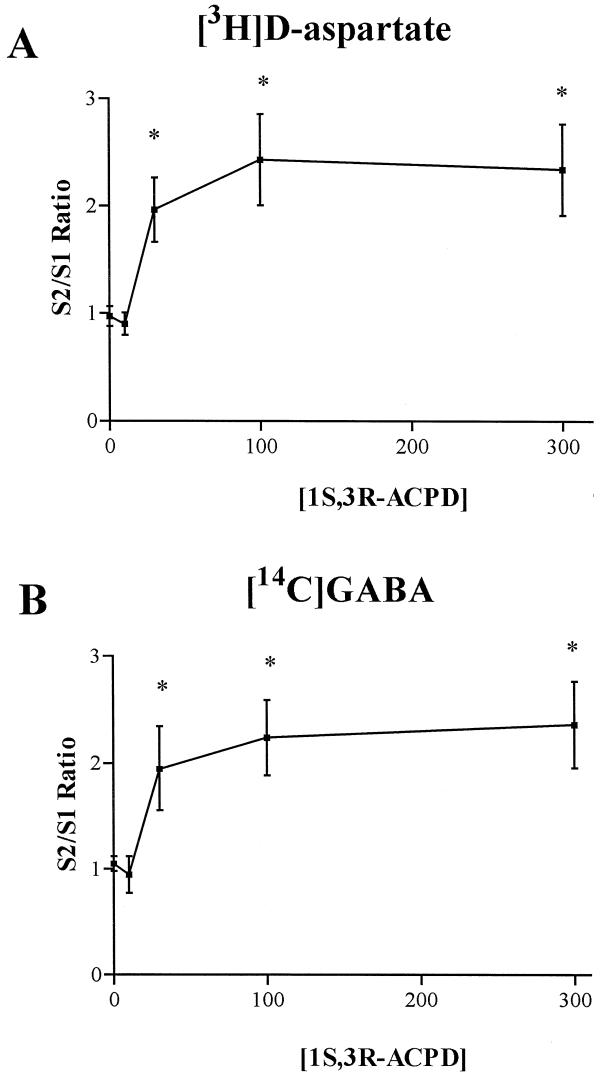
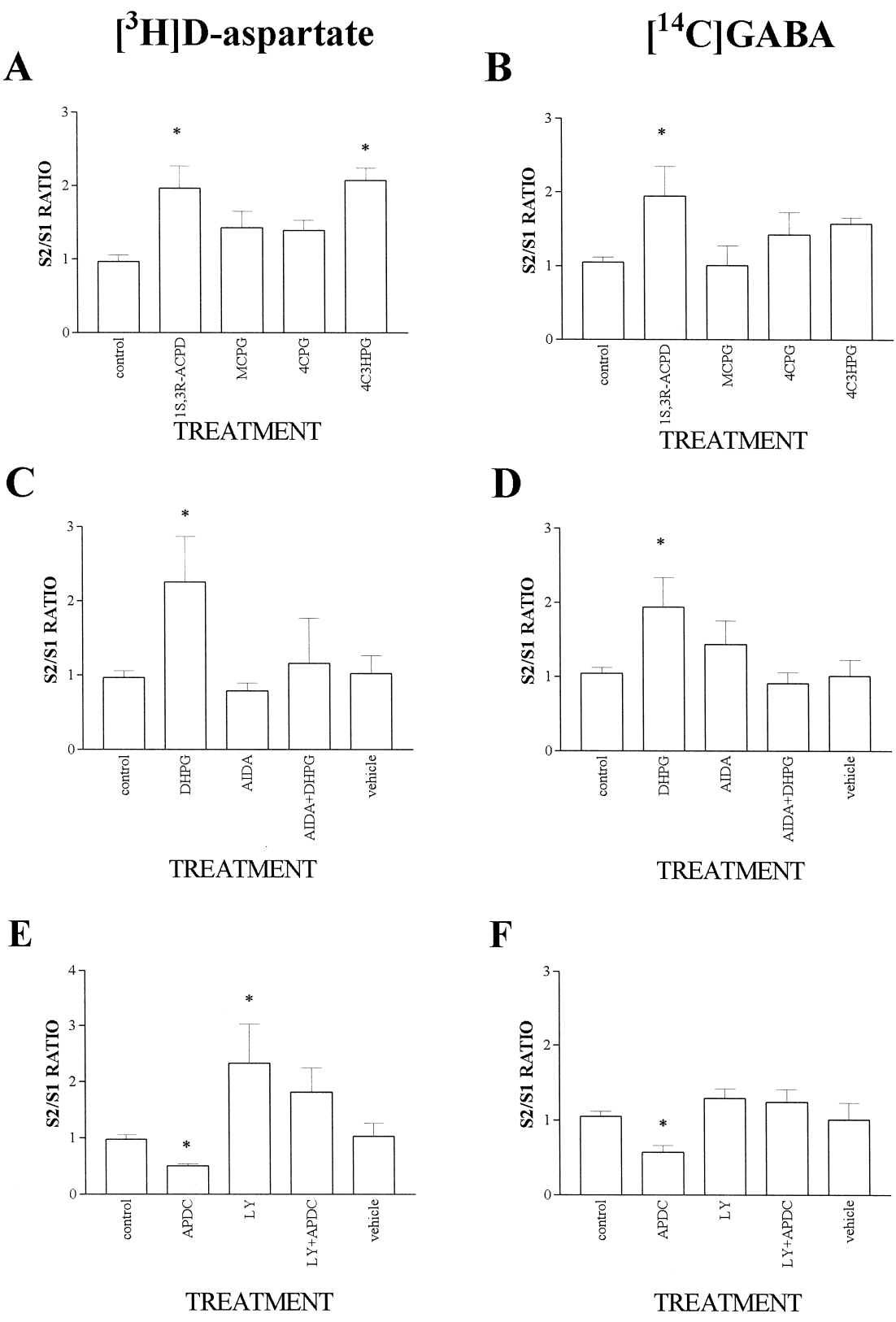


Fig. 2. 1*S*,3*R*-ACPD has a dose-dependent effect on increasing K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA. Slices of nucleus tractus solitarii, containing [^{3}H]D-aspartate and [^{14}C]GABA were exposed to two periods (2 min) of high K^+ (30 mM) stimulation (S_1 and S_2). When 1*S*,3*R*-ACPD was included in S_2, a dose-dependent (30–300 μM) increase in K^+-stimulated outflow of [^{3}H]D-aspartate (A) and [^{14}C]GABA (B), was observed. Values are shown as S_2/S_1 ratios and are the mean ± S.E.M. of 4–6 experiments. The effects of 1*S*,3*R*-ACPD which were significantly different to control values are denoted by $* P < 0.05$ (ANOVA, Dunnett's *t*-test).

and 400 μm sections were cut on a Vibratome™ at 4°C. Medial nucleus tractus solitarii (approximately 0.5 mm either side of the area postrema) was microdissected out into ice-cold buffer and these sub-sections were chopped into 200 × 200 μm prisms using a McIlwain tissue chopper.

Slices were then dispersed in 5 ml of KH at 37°C, bubbled with carbogen (95% O_2/5% CO_2), for 10 min



and rinsed by gentle centrifugation with the supernatant being removed and discarded. Nucleus tractus solitarii slices were then incubated with [^{3}H]D-aspartate (0.1 μM) and [^{14}C]GABA (0.1 μM) at 37°C for 30 min. The KH incubation buffer also contained β-alanine (1 mM), which prevents glial uptake of [^{14}C]GABA (Iversen and Kelly, 1975) and γ-vinyl GABA (100 μM), which is a GABA transaminase inhibitor, used here to prevent metabolism of [^{14}C]GABA (Jung et al., 1977). Inhibitors of glutamate transport were not employed here or during superfusion because of the difficulty of choosing selective inhibitors and because the outflow of [^{3}H]D-aspartate has been successfully studied in the absence of such inhibitors (Lombardi et al., 1994, 1996).

The incubation was terminated by gentle centrifugation as above. Slices were then re-suspended in KH buffer with gentle agitation and 200 μl of this suspension were dispersed into superfusion chambers of a Brandel Superfusion model 600 instrument and the slices superfused at a constant rate of 0.5 ml/min with KH buffer (37°C) for 75 min. During this washout period a 2 min stimulation with 30 mM K^+-enriched KH buffer was used to expedite the removal of loosely associated transmitter (Jones et al., 1995). NO-711 (1 μM) was included in the superfusion buffer throughout the washout and experimental periods (Hickling et al., 1997)—this drug blocks the neuronal GABA transporter and subsequently prevents re-uptake of released [^{14}C]GABA.

Sequential 3 min fractions were collected, and radioactivity released in each fraction was determined by scintillation spectrometry. Three fractions were collected at the beginning of each experiment and averaged to determine basal outflow (R_1) of [^{3}H]D-aspartate and [^{14}C]GABA. At the fourth fraction, slices were stimulated with high K^+ (30 mM) KH buffer for 2 min. Stimulated outflow (S_1; three to six fractions) was determined as that which occurred above basal outflow. The effect of drug solutions on basal outflow was determined by collecting three sequential fractions and taking an average (R_2). At fraction 13, slices were stimulated again, as above for 2 min. Stimulated outflow (S_2) was determined as the outflow that occurred above basal. Various mGlu receptor agonists were introduced one sample (3 min) prior to and during S_2 and antagonists, when used, were superfused two samples prior to S_2. When Ca^{2+} was omitted (with Ca^{2+} being replaced by 1 mM EDTA) from KH buffer for testing the Ca^{2+}-dependence of K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA, Ca^{2+}-free KH was superfused from sample 10 through to high K^+-stimulation at sample 13.

At the end of each experiment, slices were recovered to determine the amount of residual radioactivity. Percent fractional outflow was determined by expressing the dpm in each fraction of superfusate relative to the total dpm remaining in the prisms at that particular moment at time. Drug effects were examined by expressing the relevant fractional outflow data as R_2/R_1 and S_2/S_1 ratios. Under the experimental conditions employed radiolabelled D-aspartate and GABA will be essentially unmetabolized (Beart and McDonald, 1980; Jones et al., 1995).

2.3. Data analysis

All data were expressed as mean ± S.E.M. S_2/S_1 or R_2/R_1 ratios. A one way analysis of variance (ANOVA) was used to compare mean S_2/S_1 or R_2/R_1 ratios of the various drug treatments with the corresponding control groups. Individual differences were examined by a post hoc Dunnett's *t*-test for multiple comparisons. If data were not normally distributed as determined by Sigma Stat computer programme (Jandel Scientific), a Kruskal–Wallis One-way ANOVA was performed. A $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Characteristics of K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA

A preliminary series of experiments was performed to characterize the outflow of [^{3}H]D-aspartate and [^{14}C]GABA. The non-selective mGlu receptor agonist, 1*S*,3*R*-ACPD (10–300 μM) alone had no effect on basal outflow of [^{3}H]D-aspartate and [^{14}C]GABA ($P > 0.05$). So high K^+ (30 mM) in KH buffer was used to stimulate outflow in subsequent experiments. High K^+ stimulation produced 91% and 57% increases in [^{3}H]D-aspartate and [^{14}C]GABA outflow, respectively, over basal (Fig. 1A and B), which were reproducible with no evidence of tachyphylaxis (S_2/S_1 ratios being 0.97 ± 0.09 and 1.05 ± 0.03 for [^{3}H]D-aspartate and [^{14}C]GABA, respectively). The K^+-evoked outflow of both [^{3}H]D-aspartate and [^{14}C]GABA

Fig. 3. The effect of mGlu receptor drugs on K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA. Slices of nucleus tractus solitarii, containing [^{3}H]D-aspartate and [^{14}C]GABA, were exposed to two periods (2 min) of high K^+ (30 mM) stimulation (S_1 and S_2) in the absence (control values) or presence of various mGlu receptor drugs. Values are shown as S_2/S_1 ratios and are the mean ± S.E.M. of 4–17 experiments. tACPD (30 μM) increased the K^+-evoked outflow of [^{3}H]D-aspartate (A) and [^{14}C]GABA (B) compared to control and this enhanced outflow was attenuated by a number of phenylglycine antagonists (MCPG–200 μM; 4CPG–500 μM; 4C3HPG–500 μM). The Type I-selective agonist DHPG (300 μM) also enhanced K^+-evoked outflow of [^{3}H]D-aspartate (C) and [^{14}C]GABA (D) compared to control, and this action was blocked by AIDA (100 μM). The Type II-selective agonist APDC (300 μM) inhibited K^+-evoked outflow of [^{3}H]D-aspartate (E) and [^{14}C]GABA (F) compared to control. LY307452 (LY; 200 μM), enhanced K^+-evoked outflow of [^{3}H]D-aspartate (E) and [^{14}C]GABA (F), when used alone, and antagonized the effect of APDC, when used in combination.

was shown to be Ca^{2+}-sensitive, as when Ca^{2+}-free KH buffer was employed, the stimulated outflow was significantly reduced (S_2/S_1 ratios 0.39 ± 0.14 ($n = 3$) and 0.53 ± 0.12 ($n = 3$) for [^{3}H]D-aspartate and [^{14}C]GABA, respectively; $P < 0.05$).

Following these initial experiments, 1*S*,3*R*-ACPD was included with high K^+ in S_2 and produced a large concentration-dependent (10–300 μM; Fig. 2) increase in outflow above K^+-stimulated outflow. A submaximal concentration of 30 μM was selected for use in subsequent experiments as this enhanced K^+-stimulated outflow by 98% and 86% for [^{3}H]D-aspartate and [^{14}C]GABA, respectively (Fig. 1C and D). The submaximal increase in K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA, induced by 30 μM 1*S*,3*R*-ACPD was attenuated by the mGlu receptor phenylglycine compounds MCPG (200 μM) and 4CPG (500 μM). 4C3HPG (500 μM), on the other hand, only attenuated the 1*S*,3*R*-ACPD-induced outflow of [^{14}C]GABA (Fig. 3A and B). The phenylglycine antagonists, had no effect on basal outflow.

There was no effect of the Type I and II mGlu receptor agonist CCG-I (30–300 μM; Hayashi et al., 1992) on the basal outflow of both [^{3}H]D-aspartate and [^{14}C]GABA (Table 1; $P > 0.05$). CCG-I, at concentrations of 30 and 300 μM, had no effect on K^+-evoked outflow of [^{14}C]GABA ($P > 0.05$), while 100 μM CCG-I (S_2/S_1 ratio 1.99 ± 0.35; $P < 0.05$) significantly enhanced outflow of [^{14}C]GABA compared to controls. Both 30 μM (S_2/S_1 ratio 2.05 ± 0.61; $P < 0.05$) and 100 μM (S_2/S_1 ratio 3.06 ± 0.64; $P < 0.05$) CCG-I increased the K^+-stimulated outflow of [^{3}H]D-aspartate and 300 μM had no effect compared to control ($P > 0.05$). The inconsistencies between results of CCG-I on K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA, could possibly have been due to actions of CCG-I at Type I mGlu receptors or possibly at ionotropic NMDA receptors (Ishida et al., 1990). Due to the non-selective Type I and II mGlu receptor agonist actions of ACPD and CCG-I, and the mixed agonist and antagonist actions of the phenylglycine compounds, some newer subtype-selective compounds were tested in a subsequent series of experiments.

The Type III-selective mGlu receptor agonist L-AP4 (30–300 μM) had no effect on either basal or K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA as seen in Table 1 ($P > 0.05$). These observations would suggest that Type III mGlu receptors may not be involved in the outflow of [^{3}H]D-aspartate and [^{14}C]GABA in the rat nucleus tractus solitarii under the present experimental conditions.

Table 1
Prisms of nucleus tractus solitarii were exposed to two periods (S_1 and S_2) of high K^+-stimulation in the absence and presence of a number of different concentrations of mGlu receptor agonists in R_2 and S_2

Treatment	[^{3}H]D-aspartate		[^{14}C]GABA	
	S_2/S_1	R_2/R_1	S_2/S_1	R_2/R_1
Control	0.97 ± 0.09	1.07 ± 0.03	1.05 ± 0.07	1.10 ± 0.03
ACPD (10 μM)	0.90 ± 0.10	1.12 ± 0.02	0.94 ± 0.17	1.16 ± 0.05
ACPD (30 μM)	1.96 ± 0.30[a]	1.05 ± 0.04	1.95 ± 0.39[a]	1.15 ± 0.04
ACPD (100 μM)	2.43 ± 0.29[a]	1.09 ± 0.11	2.24 ± 0.35[a]	0.99 ± 0.06
ACPD (300 μM)	2.34 ± 0.42[a]	1.26 ± 0.09	2.36 ± 0.40[a]	1.21 ± 0.03
L-AP4 (30 μM)	0.96 ± 0.13	0.96 ± 0.05	0.81 ± 0.09	1.26 ± 0.07
L-AP4 (100 μM)	1.06 ± 0.14	0.94 ± 0.05	0.97 ± 0.12	1.22 ± 0.05
L-AP4 (300 μM)	1.21 ± 0.14	0.91 ± 0.04	1.08 ± 0.10	1.13 ± 0.06
DHPG (30 μM)	1.17 ± 0.13	0.99 ± 0.06	1.04 ± 0.11	1.19 ± 0.03
DHPG (100 μM)	1.04 ± 0.18	0.99 ± 0.04	1.04 ± 0.13	1.21 ± 0.04
DHPG (300 μM)	2.26 ± 0.61[a]	0.98 ± 0.05	1.94 ± 0.41[a]	1.17 ± 0.04
CCG-I (30 μM)	2.05 ± 0.61[a]	0.97 ± 0.05	1.31 ± 0.24	1.14 ± 0.06
CCG-I (100 μM)	3.06 ± 0.65[a]	1.02 ± 0.08	1.98 ± 0.35[a]	1.06 ± 0.04
CCG-I (300 μM)	1.13 ± 0.21	1.32 ± 0.20	1.07 ± 0.18	1.21 ± 0.09
APDC (30 μM)	1.79 ± 0.21	1.08 ± 0.04	1.38 ± 0.36	1.18 ± 0.05
APDC (100 μM)	1.37 ± 0.18	1.03 ± 0.07	1.55 ± 0.17	1.15 ± 0.08
APDC (300 μM)	0.51 ± 0.04[a]	1.06 ± 0.05	0.56 ± 0.09[a]	1.15 ± 0.04

Data show drug effects on basal (R_2/R_1) and K^+-evoked (S_2/S_1) outflow and are mean ± S.E.M. of 4–23 individual observations.
[a] Significant effect of drug in S_2 or R_2 (ANOVA, followed by Dunnett's *t*-test; $P < 0.05$).

3.2. Type I mGlu receptors and K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA

The Type I-selective mGlu receptor agonist DHPG (300 μM; Schoepp et al., 1995b) also produced an increase in the K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA (S_2/S_1 ratios being 2.26 ± 0.60 for [^{3}H]D-aspartate and 1.94 ± 0.40 for [^{14}C]GABA; $P < 0.05$) compared to control. Lower concentrations of DHPG (30–100 μM) were ineffective in altering outflow (Table 1; $P > 0.05$). The increase in K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA induced by 300 μM DHPG was reversed by the Type I-selective antagonist (Pelliciari et al., 1995) AIDA (100 μM), which had no effect on K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA when used alone (S_2/S_1 ratios: 0.79 ± 0.11 for [^{3}H]D-aspartate and 1.43 ± 0.31 for [^{14}C]GABA), but when used in combination with DHPG (300 μM), AIDA reduced K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA back to control levels (Fig. 3C and D).

3.3. Type II mGlu receptors and K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA

The recently developed Type II-selective mGlu receptor agonist APDC (300 μM; Schoepp et al., 1995a) decreased K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA (S_2/S_1 ratios being 0.51 ± 0.04 for [^{3}H]D-aspartate and 0.56 ± 0.09 for [^{14}C]GABA; $P < 0.05$) compared to control (Table 1; Fig. 3D and E). Lower concentrations of APDC (30–100 μM) were ineffective in altering outflow (Table 1; $P > 0.05$). The decrease in K^+-evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA was reversed by the type II antagonist LY307452 (Wermuth et al., 1996; 200 μM), when used in combination with APDC (300 μM)—

LY307452 reduced K^+-evoked outflow back to control levels (Fig. 3E and F). LY307452 (200 μM) alone, however, significantly enhanced K^+-stimulated outflow of [^{3}H]D-aspartate ($P < 0.05$), but not [^{14}C]GABA compared to control (S_2/S_1 ratios: 2.33 ± 0.71 for [^{3}H]D-aspartate and 1.29 ± 0.12 for [^{14}C]GABA). This effect was not due to the vehicle used to dissolve drugs, because vehicle effects on K^+-stimulated outflow (S_2/S_1 ratios: 1.03 ± 0.24 for [^{3}H]D-aspartate and 1.01 ± 0.22 for [^{14}C]GABA) were not observed under the conditions used in our experimental system (Fig. 3).

4. Discussion

The present studies suggest the existence of release-regulating mGlu receptors in rat nucleus tractus solitarii. The initial results with 1*S*,3*R*-ACPD and the mGlu receptor phenylglycine antagonists, and with CCG-I, indicate the presence of both Type I and Type II mGlu receptors in the rat nucleus tractus solitarii—given the reported pharmacological selectivity of these compounds (Thomsen et al., 1994; Roberts, 1995). MCPG has been shown to be a selective mGlu receptor antagonist at $mGlu_{1\alpha}$ receptor and $mGlu_5$ receptor subtypes (Kemp et al., 1994; Thomsen et al., 1994) and 4CPG is selective for $mGlu_1$ receptor, but not $mGlu_5$ receptor (Brabet et al., 1995). CCG-I is a mGlu receptor agonist which has been shown to have effects at both Type I and Type II mGlu receptors as well as at NMDA receptors (Hayashi et al., 1992). At higher concentrations, CCG-I acts as a Type I mGlu receptor agonist, while at lower concentrations CCG-I acts as a Type II mGlu receptor agonist (Aramori and Nakanishi, 1992; Tanabe et al., 1992). The actions of Type I and II mGlu receptors appear to be quite different in regard to modulating transmitter release (Lombardi et al., 1994, 1996), therefore, subtype-selective mGlu receptor agonists and antagonists were used to study K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA from slices of nucleus tractus solitarii and it was demonstrated that Type I mGlu receptors facilitated outflow, while Type II mGlu receptors inhibited outflow. However, the Type III-selective mGlu receptor agonist, L-AP4 had no effect on basal or K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA from slices of rat nucleus tractus solitarii, which would tend to suggest that Type III mGlu receptors are not present in this nucleus. Several groups have reported that L-AP4 has an inhibitory effect on K^+-evoked and 4-aminopyridine-induced release of glutamate in cerebrocortical (Vásquez et al., 1995) and striatal (East et al., 1995) synaptosomes.

The present study was performed to provide pharmacological insights into our previous findings from in vivo microdialysis experiments where we have evidence indicative of presynaptic mGlu receptors regulating amino acid release in nucleus tractus solitarii (Jones et al., 1998). We employed slices of nucleus tractus solitarii and examined the outflow of exogenously accumulated, radiolabelled transmitter in a model system that is amenable to the analysis of the effects of multiple mGlu receptor agonists and antagonists. Thus, while our data are indicative of the roles for Type I and II mGlu receptors, they should be interpreted with caution as the outflow may involve non-vesicular transmitter release and/or reversal of the transporter (Nicholls and Attwell, 1991; Ruzicka and Jhamandas, 1993; unlikely for the [^{14}C]GABA), although the same approach has been used successfully with [^{3}H]D-aspartate and cortical slices (Lombardi et al., 1996). However, the evoked outflow of [^{3}H]D-aspartate and [^{14}C]GABA in the present study, was markedly reduced following the removal of Ca^{2+} from the superfusion buffer, which would tend to suggest that at least a major portion of the K^+-induced increase in outflow of [^{3}H]D-aspartate and [^{14}C]GABA is neuronal in origin.

There was no increase in basal outflow of [^{3}H]D-aspartate and [^{14}C]GABA in the present experiments using an in vitro slice preparation containing nucleus tractus solitarii upon inclusion of mGlu receptor agonists. Other groups have reported that mGlu receptors can stimulate release of dopamine in vivo in striatum (Arai et al., 1996) and nucleus accumbens (Taber and Fibiger, 1995). The effect of mGlu receptors on in vitro release is commonly studied by looking at K^+- or 4-aminopyridine-evoked release, and there have been no studies reporting that mGlu receptors affect in vitro release on their own. It has been suggested that the presence of arachidonic acid is required to facilitate K^+-evoked outflow of [^{3}H]D-aspartate in the cortex (Lombardi et al., 1996), as well as Glu release following 4-aminopyridine stimulation in cortical synaptosomes (Herrero et al., 1994; Vásquez et al., 1995). Perhaps, in vivo, there are large enough quantities of arachidonic acid present to facilitate release, while in the current in vitro system there is not.

DHPG, the Type I mGlu receptor-selective agonist (Schoepp et al., 1995b), enhanced K^+-stimulated outflow of [^{3}H]D-aspartate and [^{14}C]GABA from slices of nucleus tractus solitarii, which would be consistent with reports suggesting that Type I, phosphoinositide-linked mGlu receptors are involved in facilitating neurotransmitter release (Lombardi et al., 1994, 1996). Additionally, recent evidence demonstrating that AIDA antagonized the effect of 1*S*,3*R*-ACPD on K^+-stimulated outflow of [^{3}H]D-aspartate in a rat cortical slice preparation would further implicate a role for Type I mGlu receptors in facilitation of transmitter release (Moroni et al., 1997). The present studies have also revealed the inhibitory effect of the Type I mGlu receptor antagonist, AIDA, on the DHPG enhancement of the K^+-stimulated outflow. Type I mGlu receptors have also been localized in nucleus tractus solitarii by immunocytochemistry (Romano et al., 1995; Van den Pol, 1994), which along with the present evidence suggest functional roles for this subtype of mGlu receptor in nucleus tractus solitarii.

The observation that the Type II APDC agonist (Schoepp et al., 1995a) inhibits K^+-stimulated outflow in nucleus tractus solitarii would also be consistent with previous studies suggesting that Type II–AC-linked mGlu receptors have an inhibitory effect on depolarization-induced outflow (Lombardi et al., 1994, 1996). A particularly interesting observation suggesting activation by depolarization of discrete inhibitory subpopulations of Type II mGlu receptors, was the ability of LY307452 to increase K^+-induced outflow of [^{3}H]D-aspartate. This result is suggestive of a tonic inhibition of the release process by extracellular Glu activating Type II mGlu receptors.

Like Glu, a role for GABA in the modulation of cardiovascular reflexes has been well-documented (Lawrence and Jarrott, 1996). In vitro studies in slices containing nucleus tractus solitarii have provided evidence that GABA, along with Glu, is released in a calcium-dependent manner following electrical or high K^+-stimulation (Kihara et al., 1989; Meeley et al., 1989) and microdialysis studies in nucleus tractus solitarii have shown that GABA release occurs in nucleus tractus solitarii following stimulation with high K^+ (Sved, 1994). While Glaum and Miller (1992, 1993) and Glaum et al. (1994) have demonstrated that mGlu receptors regulate the activity of glutamatergic and GABAergic neurones in solitary nucleus, our in vitro release studies provide new evidence for the specific involvement of Type I and II mGlu receptors in this modulation in nucleus tractus solitarii. Although it is difficult to extrapolate from our in vitro experiments to the physiological situation, the current findings, along with these ex vivo electrophysiological studies and our own functional studies demonstrating that Type I and II mGlu receptor agonists produce depressor and bradycardic responses (Jones et al., 1996), suggest roles for mGlu receptors in the processing of cardiovascular information in nucleus tractus solitarii.

Acknowledgements

This work was supported by grants from the Ramaciotti Foundation and a Small Grant from the Australian Research Council. Thank you to Dr. D.D. Schoepp for continued interest. Gifts of drugs were greatly appreciated.

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