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Type I and II metabotropic glutamate receptors mediate depressor and bradycardic actions in the nucleus of the solitary tract of anaesthetized rats

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Abstract

The potential role of metabotropic glutamate (mGlu) receptors in cardiovascular function in the nucleus of the solitary tract was examined following the microinjection of a number of selective mGlu receptor compounds into this site of anaesthetized rats. The prototypic mGlu receptor selective agonist 1*S*,3*R*-1-amino-cyclopentane dicarboxylate elicited depressor and bradycardic actions following microinjection into the nucleus tractus solitarius, which were similar to those produced by L-glutamate. Similarly, decreases in blood pressure and heart rate were observed upon administration of the type I and II selective mGlu receptor agonists, (*R*,*S*)-3,5-dihydroxyphenylglycine (DHPG) and 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (APDC), respectively. These actions of DHPG were selectively attenuated by (±)-1-aminoindane-1,5-dicarboxylate, a type I mGlu receptor antagonist, whilst cardiovascular responses to APDC were unaffected by this compound. Interestingly, the proposed type II antagonist, (2*S*,4*S*)-2-amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-doic acid, reduced the cardiovascular responses to intra-nucleus tractus solitarius administration of both APDC and DHPG. The type III mGlu receptor agonist, L-2-amino-4-phosphonobutyrate, however, failed to elicit any cardiovascular actions when microinjected into the nucleus tractus solitarius. These studies provide new evidence for functional type I and II mGlu receptors in modulating cardiovascular responses in the nucleus tractus solitarius.

Keywords: Metabotropic glutamate receptor; Nucleus tractus solitarii; Blood pressure; Heart rate; Glutamate

1. Introduction

The nucleus of the solitary tract is the site of termination for a number of populations of primary afferent neurons, including baroreceptor afferents projecting from the aortic arch and carotid sinus (Kumada et al., 1990). Given this evidence, it is not surprising that the nucleus tractus solitarius has an important role in central regulation of blood pressure, via involvement in the arterial baroreceptor reflex. A number of different neurotransmitters and neuromodulators have been shown to modulate cardiovascular activity within the nucleus tractus solitarius (Lawrence and Jarrott, 1996). Indeed, a major role for the excitatory amino acid, L-glutamate (Glu) in central cardiovascular control has been well established, with evidence supportive of Glu being the primary neurotransmitter at baroreceptor afferent neurones (Lawrence and Jarrott, 1996). Additionally, two other major projections involved in the baroreflex pathway are also glutamatergic (Arnolda et al., 1992). These projections exist from the nucleus tractus solitarius to caudal ventrolateral medulla and from the rostral ventrolateral medulla to the intermediolateral column of the spinal cord.

Glu receptors have been reported to be present on baroreceptor afferent terminals in the nucleus tractus solitarius (Lewis et al., 1988) and transmitter-like release of Glu occurs following electrical stimulation of the vagus nerve (Allchin et al., 1994) and baroreceptor activation after administration of phenylephrine (Lawrence and Jarrott, 1994). Injection of Glu into the nucleus tractus solitarius of anaesthetized rats induces cardiovascular responses, which are similar to the depressor and bradycardic responses elicited by baroreflex activation (Talman et al., 1980), and are considered to be due to activation of

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ion-channel gated Glu receptors. Likewise, microinjections of the ionotropic Glu receptor agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate into the nucleus tractus solitarius cause decreases in blood pressure and heart rate which mimic the response to Glu. Interestingly, the ionotropic Glu receptor antagonist, kynurenate attenuated the cardiovascular responses to NMDA, AMPA and kainate, but not those of Glu (Leone and Gordon, 1989) suggesting a non-ionotropic Glu component of action in this nucleus.

In addition to the ion-channel gated Glu receptors, a family of G-protein-coupled metabotropic glutamate (mGlu) receptors have been identified, which are linked to a number of second messenger systems, including calcium mobilization, phosphoinoside hydrolysis and cyclic AMP accumulation (Conn and Pin, 1997). Recent evidence provided by molecular cloning studies has indicated the presence of at least eight subtypes of mGlu receptors, with a number of spliced variants having also been described. Pharmacologically, mGlu receptors can be distinguished from ionotropic Glu receptors by using the mGlu receptor selective agonists (±)1*S*,3*R*-1-amino-cyclopentane-1,3-dicarboxylic acid (tACPD) and L-2-amino-4-phosphonobutyrate (L-AP4). Moreover, the G-protein coupled mGlu receptors may also have roles in cardiovascular regulation in the nucleus tractus solitarius as injections of tACPD into the nucleus tractus solitarius produce dose-dependent depressor and bradycardic responses in anaesthetized rats (Pawloski-Dahm and Gordon, 1992). This same study also showed that L-AP3, a non-selective mGlu receptor antagonist, decreased blood pressure and heart rate following microinjection into the nucleus tractus solitarius.

The electrophysiological studies of Glaum and Miller (1992) demonstrated that, in slices of coronal brainstem, 1*S*,3*R*-ACPD can directly depolarize solitary neurones, in addition to having other pre- and postsynaptic actions at amino acid-utilizing neurones within the nucleus tractus solitarius. These actions of 1*S*,3*R*-ACPD on solitary neurones were blocked by a number of mGlu receptor phenylglycine antagonists, some of which also elicited agonist-like responses (Glaum et al., 1993). We have previously described evidence for functional mGlu receptors in the nucleus tractus solitarius which can modulate the release in vivo and in vitro of various amino acids (Jones et al., 1998a,b). In particular, the in vitro release experiments in the nucleus tractus solitarius slice preparation have shown that both type I and II mGlu receptors are present via the use of subtype-specific mGlu receptor agonists and antagonists (Jones et al., 1998b).

The aim of the present study was to examine roles for the different subtypes of mGlu receptors in cardiovascular processing in nucleus tractus solitarius by microinjecting a number of mGlu receptor agonists and antagonists into the nucleus tractus solitarius of anaesthetized rats and monitoring blood pressure and heart rate responses.

2. Materials and methods

2.1. Surgical procedures

Experiments were performed in accordance with the NHMRC Code of practice for the care and use of animals for experimental purposes in Australia. Male Sprague–Dawley rats (280–400 g) were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.) and catheters were inserted into the carotid artery and jugular vein for blood pressure monitoring and drug administration, respectively. Pulsatile blood pressure was monitored via a pressure transducer connected to a polygraph (Grass Instruments, Quincy, MA, USA) or a MacLab System (ADInstruments, Sydney, Australia), and mean arterial pressure and heart rate were derived from the phasic signal.

Rats were placed in a stereotaxic frame (Stoelting, IL, USA), the skull surface exposed and a stainless steel guide cannula (24-gauge, 14 mm long) inserted at the following stereotaxic coordinates: AP −13.3; L +0.9; V −5.8; incisor bar −3.3 mm (taken from bregma; Paxinos and Watson, 1991). Following implantation of the guide cannula, which was cemented to the skull with dental acrylic (Vertex, Holland), the rat was allowed to stabilise after surgery for 1 h.

2.2. Nucleus tractus solitarius injections

For microinjection of drugs, a stainless steel injector (31-gauge, 16 mm long) was inserted through the guide cannula. Each injector was attached via polyethylene tubing to a microsyringe (Scientific Glass Engineering, Melbourne, Australia) and mGlu receptor drugs (in artificial cerebrospinal fluid) were injected unilaterally in a volume of 100 nl over a time period of 30 s while blood pressure and heart rate were recorded continuously. Anaesthesia was supplemented during the experiment, as required, but at least 10 min was allowed before any subsequent mGlu receptor drug administration.

In order to identify injection sites, brilliant blue dye (100 nl) was injected at the conclusion of experiments. Following this procedure, animals were given an overdose of pentobarbitone sodium (i.v.), brains were removed and the brainstem rapidly dissected free and frozen in liquid nitrogen. Frozen sections (14 μm) were then cut on a cryostat and sections were stained with thionin and observed under a light microscope for identification of injection sites (Fig. 1).

When mGlu receptor agonists were injected into the nucleus tractus solitarius, cardiovascular responses normally lasted in the order of 1–2 min. No more than three different drugs or three doses of the same compound were injected into the same rat, and at least 30 min was allowed before injection of the next agonist/dose. For antagonist studies, the appropriate agonist was initially injected at

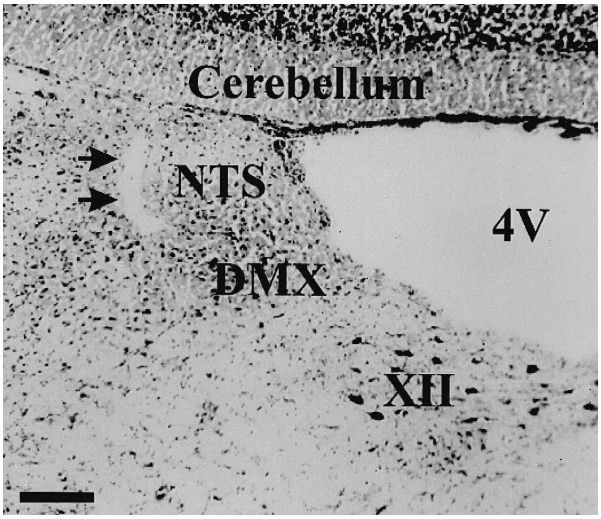

Fig. 1. A representative photomicrograph of a 14-μm thick, thionin-stained brainstem section (scale bar = 100 μm) showing the microinjection site of the nucleus tractus solitarius (NTS). This micrograph illustrates that the injector site is within the NTS, and also shows the areas of cerebellum, 4th ventricle (4V), dorsal motornucleus of the vagus (DMX) and hypoglossal nucleus (XII).

least 30 min before the antagonist was given, and the agonist was injected again 5 min after the antagonist while cardiovascular variables were being monitored. In a limited number of cases, agonists were also re-tested 30 min after antagonist injection to determine duration of action of antagonists.

2.3. Data analysis

Data are expressed as mean ± S.E.M. of absolute mean arterial pressure and heart rate or as changes in these parameters. The effects of individual mGlu receptor compounds vs. pre-drug baseline values were compared using Student's paired *t*-test, while one-way analysis of variance (ANOVA) was performed to examine agonist/antagonist effects, with post-hoc testing using Student Newman–Keuls test. All statistical tests were performed on a commercially available statistical package (Sigmastat, Jandel Scientific, USA) and a P value < 0.05 was considered to be statistically significant.

2.4. Materials

1*S*,3*R*-ACPD, L-AP4, (+)-α-methyl-4-carboxyphenylglycine (MCPG), (*R*,*S*)-3,5-dihydroxyphenylglycine (DHPG) and (±)-1-aminoindan-1,5-dicarboxylic acid (AIDA) were purchased 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). When appropriate, 1:1 equivalents of NaOH was employed to aid

Table 1
Effect of mGlu receptor drugs on mean blood pressure and heart rate following microinjection into the nucleus tractus solitarius of anaesthetized rats
Values are mean ± S.E.M. of $n = 3$–8 rats and represent basal levels before and at the time of maximal change.

Treatment	Dose (pmol)	Mean blood pressure (mmHg)		Heart rate (bpm)	
		Before	After	Before	After
GLU	300	118 ± 7	94 ± 7[a]	333 ± 23	216 ± 58[a]
MCPG	200	101 ± 9	66 ± 6[a]	380 ± 23	349 ± 20[a]
L-AP4	300	95 ± 10	88 ± 11	432 ± 26	431 ± 26
ACPD	30	107 ± 9	84 ± 15	416 ± 20	389 ± 33
ACPD	100	105 ± 7	63 ± 8[a]	355 ± 24	320 ± 17
ACPD	300	106 ± 8	52 ± 3[a]	460 ± 36	393 ± 32[a]
DHPG	30	103 ± 5	59 ± 3[a]	413 ± 15	373 ± 20[a]
DHPG	60	107 ± 7	68 ± 15[a]	415 ± 22	377 ± 41[a]
DHPG	100	97 ± 3	60 ± 5[a]	410 ± 26	370 ± 30[a]
APDC	100	100 ± 6	83 ± 12	403 ± 18	390 ± 21
APDC	300	123 ± 8	92 ± 10[a]	467 ± 26	410 ± 26[a]
AIDA	100	104 ± 4	96 ± 5	449 ± 24	436 ± 18
LY307452	200	104 ± 4	92 ± 10	482 ± 24	455 ± 24

[a] $P < 0.05$ relative to control (before values) (paired *t*-test).

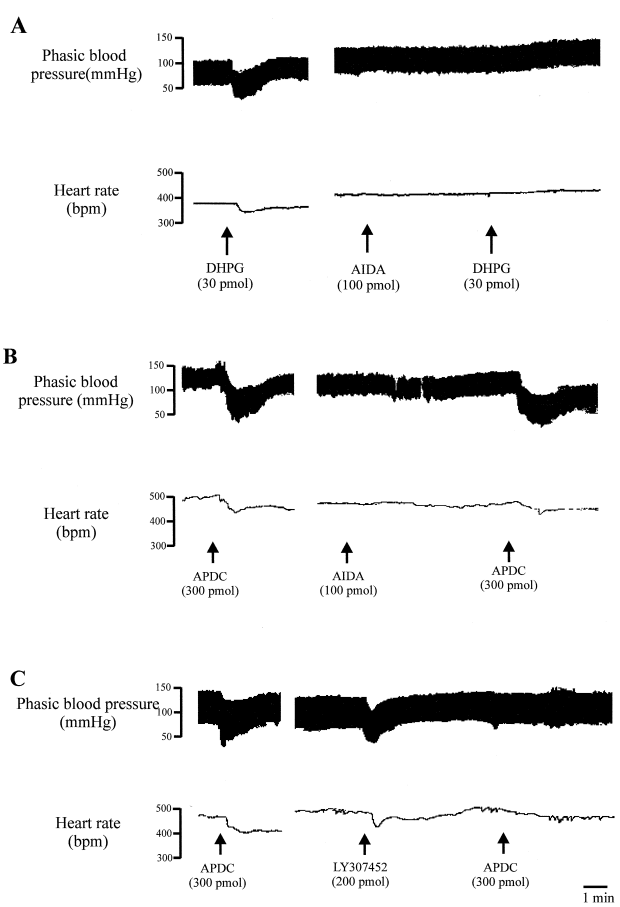

Fig. 2. Typical traces of phasic blood pressure and heart rate following microinjections into the nucleus tractus solitarius of (A) DHPG (30 pmol) before and after administration of AIDA (100 pmol), APDC (300 pmol) before and after administration of (B) AIDA (100 pmol) and (C) LY307452 (200 pmol).

drug solubilization, followed by further dilutions in artificial cerebrospinal fluid (composition in mM: glucose, 5; NaCl, 125; $NaHCO_3$, 27; KCl, 2.5; NaH_2PO_4, 0.5; Na_2HPO_4, 1.2; $NaSO_4$, 0.5; $MgCl_2$, 1; $CaCl_2$, 1).

3. Results

3.1. Preliminary characterization: Glu and mGlu receptor compounds

The effects of the unilateral microinjection into the nucleus tractus solitarius of Glu and a number of selective and non-selective mGlu receptor drugs were examined in anaesthetized rats (Table 1). There were no significant differences in the basal mean arterial pressure values prior to drug injections ($P > 0.05$, ANOVA). However, there was a range of 149 bpm in basal heart rate values prior to drug injections (Table 1) which resulted in a significant difference in basal heart rate values between animals ($P < 0.05$, ANOVA).

Glu (300 pmol) produced large decreases in mean arterial pressure (-24 ± 3 mm Hg, $n = 7$, $P < 0.05$) and heart rate (-117 ± 39 bpm, $n = 7$, $P < 0.05$), following microinjection into the nucleus tractus solitarius (Table 1). These depressor and bradycardic responses were significantly different from vehicle control values (-2 ± 1 mm Hg ($n = 6$) and 3 ± 4 bpm ($n = 6$), respectively), illustrating that microinjection of artificial cerebrospinal fluid was without effect.

The prototypical mGlu receptor agonist, 1*S*,3*R*-ACPD (30–300 pmol), also decreased mean arterial pressure and heart rate (Table 1). 1*S*,3*R*-ACPD, at 100 and 300 pmol, induced depressor responses of -41 ± 6 mm Hg ($n = 7$) and -54 ± 10 mm Hg ($n = 6$), while only the highest dose of 1*S*,3*R*-ACPD significantly decreased heart rate (-67 ± 13 bpm ($n = 6$), respectively. In addition, nucleus tractus solitarius injections of MCPG (200 pmol), a purported mGlu receptor phenylglycine antagonist at mGlu1α

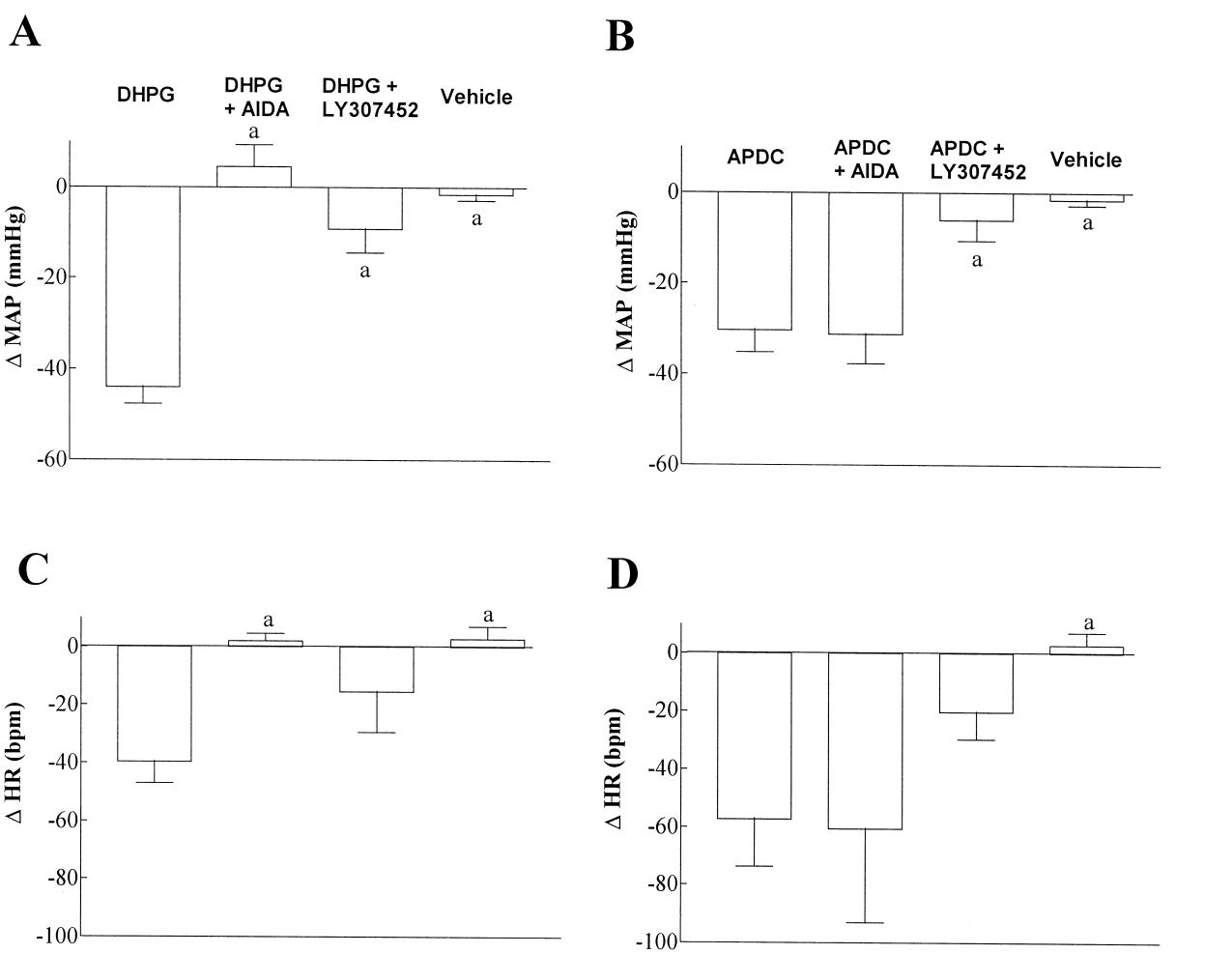

Fig. 3. Changes in (A) and (B) mean arterial pressure, and (C) and (D) heart rate following the microinjection of (A) and (C) DHPG (30 pmol), and (B) and (D) APDC (300 pmol) before and after the administration of type I and II mGlu receptor antagonists AIDA (100 pmol) and LY307452 (200 pmol), respectively, into the rat nucleus tractus solitarius. Data are presented as mean ± S.E.M. from 4–8 individual animals. [a]$P < 0.05$, significantly different compared with agonist alone (ANOVA, Newman–Keuls test).

receptor and mGlu2 receptor (Kemp et al., 1994; Thomsen et al., 1994), caused depressor (-35 ± 4 mm Hg, $n = 4$) and bradycardic (-31 ± 10 bpm, $n = 4$) responses, whereas the type III mGlu receptor agonist L-AP4 (300 pmol; $n = 3$), had no effect on mean arterial pressure or heart rate following microinjection into the nucleus tractus solitarius (Table 1).

3.2. Type I mGlu receptors: DHPG

Given the observed agonist-like actions of MCPG, together with the fact that 1*S*,3*R*-ACPD can act at both type I, PI-linked and type II, AC-linked mGlu receptors (Palmer et al., 1989), another two series of experiments were performed in order to examine some of the newer subtype-selective mGlu receptor agonists and antagonists.

DHPG (30–100 pmol), a type I-selective mGlu receptor agonist (Schoepp et al., 1994), significantly decreased mean arterial pressure and heart rate when microinjected into the nucleus tractus solitarius, although there was no dose-dependency at the doses tested (Table 1). The effects of the selective, type I and type II mGlu receptor antagonists, AIDA (100 pmol; Pellicciari et al., 1995) and LY307452 (200 pmol; Wermuth et al., 1996), respectively, were tested against DHPG (30 pmol) in separate groups of rats. Both of the mGlu receptor antagonists had no significant effects on mean arterial pressure or heart rate when microinjected alone (Table 1, Fig. 2). As expected, the depressor and bradycardic responses evoked by DHPG were abolished when microinjected 5 min after the type I antagonist AIDA (Figs. 2 and 3). However, the type II antagonist, LY307452 also significantly reduced the depressor action of DHPG (Fig. 3). The antagonism caused by both AIDA and LY307452 was reversible, as the depressor and bradycardic responses after microinjection of DHPG returned to near control values (approximately -36 mm Hg and -43 bpm) when re-tested in two animals 30 min after antagonist administration.

3.3. Type II mGlu receptors: APDC

The type II mGlu receptor agonist, APDC (Schoepp et al., 1995), was microinjected into the nucleus tractus solitarius at 2 doses (100 and 300 pmol). APDC decreased mean arterial pressure and heart rate, although only the effects at the higher dose achieved significance (Table 1, Fig. 2). The depressor and bradycardic actions of APDC (300 pmol) were attenuated when microinjected 5 min after the type II antagonist LY307452, although only the reduction in mean arterial pressure was significant (Figs. 2 and 3). Moreover, the antagonism of APDC by LY307452 was reversible, as the depressor and bradycardic effects of APDC returned to near control values (approximately -22 mm Hg and -41 bpm) in two animals tested 30 min after antagonist administration. By contrast, the APDC-induced reductions in mean arterial pressure and heart rate were not altered by the type I antagonist AIDA (Figs. 2 and 3).

4. Discussion

The present studies were designed to investigate possible roles for mGlu receptor subtypes in cardiovascular control in the nucleus tractus solitarius of anaesthetized rats. It should be noted that basal heart rate and, to a lesser extent, mean arterial pressure values varied between animals, which most likely reflects the difficulty in matching levels of anaesthesia, and hence autonomic control, between anaesthetised animals. Nevertheless, these are the first in vivo studies to report that both type I and II mGlu receptors exert cardiovascular effects following microinjection of a number of subtype-selective mGlu receptor agonists and antagonists into the nucleus tractus solitarius.

Glu is known to produce both depressor and bradycardic effects following microinjection into the nucleus tractus solitarius of anaesthetized rats (Talman et al., 1980), and this effect was confirmed in the present study. Given that Glu acts at both ionotropic Glu receptors and mGlu receptors, we used 1*S*,3*R*-ACPD as the prototypical agonist selective for mGlu receptors over ionotropic Glu receptors (Palmer et al., 1989). Microinjection of this compound into the nucleus tractus solitarius of anaesthetized rats caused depressor and bradycardic effects with the hypotensive action being dose-dependent, which is in agreement with the cardiovascular effects of nucleus tractus solitarius injections of the racemate, tACPD (Pawloski-Dahm and Gordon, 1992). Taken together with other studies describing electrophysiological (Glaum and Miller, 1992), microiontophoretic (Zhang and Mifflin, 1997) and respiratory (Vitagliano et al., 1995; DiMicco and Monroe, 1996) effects of 1*S*,3*R*-ACPD injected into nucleus tractus solitarius, the present study provides further evidence for the existence of mGlu receptors in the nucleus tractus solitarius, which could have possible roles in cardiovascular regulation. Additionally, cardiovascular effects of 1*S*,3*R*-ACPD have also been reported following microinjection into other cardiovascular-related nuclei, including rostral ventrolateral medulla and caudal ventrolateral medulla (Tsuchihashi and Averill, 1993; Tsuchihashi et al., 1994; Arnolda et al., 1996). In contrast, microinjection of lower doses of 1*S*,3*R*-ACPD had no effect on mean arterial pressure or heart rate when directly microinjected into intermediolateral column of the thoracic spinal cord of anaesthetized rats (Arnolda et al., 1996).

Interestingly, the type III selective mGlu receptor agonist, L-AP4, had no effect at the dose tested. This lack of effect was not due to a lower basal mean arterial pressure in this group, since DHPG (100 pmol) evoked a marked fall in mean arterial pressure despite a similar basal mean arterial pressure (Table 1). This finding suggests that the type III, L-AP4-sensitive mGlu receptors may not be involved in cardiovascular processing within the nucleus tractus solitarius, which is in agreement with previous studies showing a lack of effect L-AP4 on cardiovascular function (Leone and Gordon, 1989) and on in vitro release

of either [^{3}H]D-ASP or [^{14}C]gamma-aminobutyric acid from nucleus tractus solitarius slices (where a range of doses was tested) (Jones et al., 1998b). On the other hand, the mGlu receptor phenylglycine antagonist, MCPG (Kemp et al., 1994; Thomsen et al., 1994), itself evoked a depressor and bradycardic response, which was similar to the agonist actions observed for microinjection of Glu and 1*S*,3*R*-ACPD. These unexpected actions of MCPG could possibly reflect the lack of selectivity of this compound for the different subtypes of mGlu receptors (Kemp et al., 1994; Thomsen et al., 1994) or also mixed agonist/antagonist actions. Agonist actions of MCPG, reported from electrophysiological studies in amygdala and in hippocampus of the rat (Keele et al., 1995; Breakwell et al., 1998), would support this latter view.

Previous studies have failed to block the cardiovascular effects of 1*S*,3*R*-ACPD injected in the rostral ventrolateral medulla using L-AP3 or phenylglycine derivatives (Tsuchihashi and Averill, 1993; Tsuchihashi et al., 1994). Therefore, due to the potential actions of 1*S*,3*R*-ACPD and MCPG at multiple subtypes of mGlu receptors, a newer generation of subtype-selective mGlu receptor agonists was examined. DHPG, the type I selective mGlu receptor agonist (Schoepp et al., 1994), decreased mean arterial pressure and heart rate although these effects were not dose-dependent. Compared to 1*S*,3*R*-ACPD, DHPG is a more potent agonist at type I mGlu receptors (Ito et al., 1992), perhaps explaining the marked effect even at the lower doses of DHPG. The cardiovascular effects of DHPG were abolished by the type I-selective antagonist AIDA (Pellicciari et al., 1995; Moroni et al., 1997), indicating a major role for type I mGlu receptors. A confounding factor in this interpretation was that LY307452 also antagonized the actions of DHPG even though LY307452 was initially shown to have no effect on 1*S*,3*R*-ACPD-induced phophoinositide hydrolysis in cell lines (Wermuth et al., 1996). However, given that LY307452 was reported to antagonize the effects of 1*S*,3*R*-ACPD on PI hydrolysis in rat hippocampal slices (Schoepp et al., 1996), our data with DHPG suggests that LY307452 may act at type I and II mGlu receptors.

The type II mGlu receptor agonist APDC (Schoepp et al., 1995) also caused depressor and bradycardic responses after injection into the nucleus tractus solitarius. APDC has a potency similar to 1*S*,3*R*-ACPD at type II mGlu receptors, whilst showing no activity at the type I mGlu receptors in rat hippocampal slices as well as mGlu receptor-expressing non-neuronal cell lines (Schoepp et al., 1995). In the present study, the cardiovascular effects of APDC were antagonized by LY307452, but were unaffected by AIDA, suggesting that the cardiovascular effects of APDC were in fact mediated by type II mGlu receptors. These data confirm our previous in vitro release data demonstrating the presence of functional type I and II mGlu receptors in the nucleus tractus solitarius (Jones et al., 1998b). However, the greater efficacy of DHPG relative to 1*S*,3*R*-ACPD and APDC is suggestive of a greater role being played by type I mGlu receptors.

Finally, it should be noted that the nucleus tractus solitarius is a complex structure which also receives many non-cardiovascular afferent inputs, and that different baselines between anaesthetised animals could possibly have influenced the magnitude of cardiovascular changes observed following injections of mGlu receptor compounds. Therefore, the present studies are noteworthy since reproducible cardiovascular responses were obtained with the in vivo activities of the mGlu receptor compounds tested generally paralleled by their in vitro selectivities. Moreover, we have established the presence of functional type I and II mGlu receptors within the nucleus tractus solitarius and confirmed that AIDA is a highly selective type I antagonist because it attenuated the response to DHPG but not APDC, whereas the selectivity of LY307452 appears to be less clear cut. While the present study further demonstrates a role for mGlu receptors in viscerosensory processing, both mGlu receptor antagonists, at doses that attenuated their subtype-selective agonists, did not affect resting blood pressure and heart rate, suggesting that mGlu receptors do not have a tonic involvement in cardiovascular regulation in the nucleus tractus solitarius. Clearly, future studies should address whether or not either subtype I or II mGlu receptors are involved in modulation of baroreflex function at the level of the nucleus tractus solitarius.

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