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Type I and II metabotropic glutamate receptor agonists and antagonists evoke cardiovascular effects after intrathecal administration in conscious rats

[1]Xiao C. Li, *,[1]Philip M. Beart, [2]James A. Monn, [1]Nicole M. Jones & [1]Robert E. Widdop

[1]Department of Pharmacology, Monash University, Clayton, Victoria, VIC 3168, Australia and [2]Eli Lilly, Indianapolis, Indiana, IN 46285, U.S.A.

1 In the present study, the role of metabotropic glutamate receptors (mGluRs) in central cardiovascular regulation in conscious rats was examined. To this end, agonists and antagonists for type I and II mGluRs were administered intrathecally, and the temporal changes in blood pressure and heart rate were recorded.

2 L-glutamate (1 μmol) and the prototypical mGluR agonist (*1S,3R*)-ACPD (0.1 and 0.3 μmol) both increased mean arterial pressure (MAP) and heart rate (HR), implicating functional mGluRs in the spinal cord. The type I mGluR agonist DHPG (0.01–0.1 μmol) evoked increases in MAP (max = 25 ± 5 mmHg) and HR (max = 88 ± 23 beats min^{-1}). The duration of action, but not the maximum effects, were dose-related and ranged from approximately 10 min to <90 min and 1 min to >90 min for MAP and HR, respectively.

3 The type I/II mGluR agonist CCG-1 (0.1 and 0.3 μmol) caused smaller, variable increases in MAP and HR of intermediate duration (5–20 min), whereas the type II MGluR agonist APDC (0.1 and 1.0 μmol) caused marked, but transient (3–5 min), pressor and tachycardic responses. The highest doses of DHPG and CCG-1, but not APDC, also evoked behavioural responses similar to a spontaneous nociceptive behavioural effect reported previously.

4 The type I and II mGluR antagonists (AIDA and LY307452, respectively) were also given approximately 5 min before the administration of the respective type I and II mGluR agonists (DHPG and APDC). Both compounds caused pressor and tachycardic responses, with the effect of AIDA, but not LY307452, returning to control levels before mGluR agonist administration. AIDA significantly attenuated the overall cardiovascular effects of DHPG, while LY307452 significantly attenuated the overall cardiovascular effects of APDC.

5 These results indicate that functional type I and II mGluRs exist in the spinal cord, and that their activation evokes prolonged cardiovascular effects.

Keywords: Metabotropic glutamate receptors; spinal cord; intrathecal; conscious rat

Abbreviations: AIDA, (±)-1-aminoindan-1,5-dicarboxylic acid; AMPA, α-amino-3-hydroxy-5-methylisoxazolepropionate; APDC, 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate; CCG-1, (2*S*,3*S*,4*S*)-2-carboxycyclopropyl)glycine; CVLM, caudal ventral lateral medulla; DHPG, (*RS*)-3,5-dihydroxyphenylglycine; Glu, L-glutamate; HR, heart rate; iGluR, ionotropic glutamate receptor; IML, intermediolateral cell column; LY307452, (2*S*,4*S*)-2-amino-4(4,4-diphenylbut-l-yl))-pentane-1,5-dioic acid; MAP, mean arterial blood pressure; mGluR, metabotropic glutamate receptor; NMDA, N-methyl-D-aspartate; NTS, nucleus of solitary tract; rostral ventrolateral medulla; tACPD, trans-l-aminocyclopentane-1,3-dicarboxylate

Introduction

A considerable body of evidence has established that L-glutamate (Glu), the major excitatory transmitter of the central nervous system, plays a key role in the coordination of the brain's responses to changes in blood pressure and heart rate (Dampney, 1994; Sun, 1995; Lawrence & Jarrott, 1996). Thus Glu is a transmitter of vagal baroreceptor and cardiopulmonary afferent neurones, and chemoreceptor afferents, which have their terminals in the nucleus of the solitary tract (NTS). Glu is additionally a transmitter of other neuronal pathways responsible for the regulation of sympathetic nerve activity and cardiovascular functions, including a projection from NTS to the caudal ventrolateral medulla (CVLM), and reticulospinal neurones descending from the rostral ventrolateral medulla (RVLM) to the intermediolateral cell column (IML) of spinal cord. These three glutamatergic pathways (plus a likely GABAergic projection from CVLM to RVLM) are a key component of the baroreceptor reflex arc, which plays a major role in re-setting cardiovascular reflexes and hence determining ambient blood pressure and heart rate (Dampney, 1994; Sun, 1995; Lawrence & Jarrott, 1996).

The involvement of Glu in central autonomic regulation, as investigated using pharmacological agents, has been defined especially with selective agonists (N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainate) and antagonists active at the ionotropic subtypes of Glu receptors (iGluRs) (Dampney, 1994; Sun, 1995; Lawrence & Jarrott, 1996). Recent evidence has established the existence of a second major family of Glu receptors, the metabotropic receptors (mGluRs), which can regulate synaptic signalling both pre- and postsynaptically by influencing intracellular processes *via* G-proteins (Schoepp, 1994; Nicoletti *et al*., 1996; Conn & Pin, 1997). Three groups of mGluRs have been defined according to their respective transduction mechanisms, sequence homologies and pharmacological characteristics:

* Author for correspondence; E-mail: phil.beart@med.monash.edu.au

type I receptors (mGluR1,5) linked to stimulation of the phosphoinositide cascade, and type II (mGluR2,3) and type III receptors (mGluR4,6,7,8), both of which inhibit adenylyl cyclase when activated although they display different agonist and antagonist selectivities. The existence of this family of mGluRs offers additional physiological mechanisms, presumably also functional in central autonomic regulation, whereby the brain's major excitatory transmitter can subtly modulate cellular communication (Shoepp, 1994; Nicoletti *et al*., 1996; Conn & Pin, 1997). Indeed, the mGluR agonist, trans-1-aminocyclopentane-1,3-dicarboxylate (tACPD) when microinjected into the NTS produces dose-dependent depressor and bradycardic responses similar to those elicited by Glu itself (Pawloski-Dahm & Gordon, 1992; Jones *et al*., 1999) – moreover these responses are insensitive to the iGluR antagonist kynurenate indicating the involvement of mGluRs. Electrophysiological observations *ex vivo* in slices of NTS also support roles for pre- and postsynaptically located mGluRs, some of which appear to be associated with vagal afferents (Glaum & Miller 1992; 1993; Glaum *et al*., 1993). mGluRs are additionally likely to have cardiorespiratory (Vitagliano *et al*., 1994) and chemoreceptor (Vardhan *et al*., 1993a,b) roles in the NTS suggesting a wider involvement in central autonomic mechanisms. Other evidence suggests roles for mGluRs of CVLM and RVLM (Tsuchihashi & Averill, 1993; Tsuchihashi *et al*., 1994; 1997) in the sympathetic baroreflex circuitry, and possibly in respiratory regulation (Vitagliano *et al*., 1994; Li & Nattie, 1995).

As yet there have been no reports of whether or not mGluRs play functional roles *in vivo* in autonomic regulation in the IML of thoracolumbar spinal cord. The IML receives an apparently monosynaptic projection from the RVLM of vasomotor neurones which innervate sympathetic preganglionic neurones (Ross *et al*., 1981; Polson *et al*., 1992). Whilst reticulospinal neurones utilize diverse neurochemicals, Glu is well documented as their principal transmitter since (1) RVLM neurones contain Glu (Morrison *et al*., 1991; Llewellyn-Smith *et al*., 1992) and its biosynthetic enzyme phosphate-activated glutaminase (Minson *et al*., 1991), and synapse on preganglionic neurones in IML (Llewellyn-Smith *et al*., 1992), (2) Glu excites sympathetic preganglionic neurones (Backman & Henry, 1983) and the postsynaptic potentials are abolished by iGluR antagonists (Inokuchi *et al*., 1992), (3) activation of the pathway increases the release of Glu (Kapoor *et al*., 1992), and (4) intrathecal iGluR antagonists reduce sympathetic nerve discharge, arterial pressure and baroreceptor-heart rate reflex (Guyenet *et al*., 1987; Sundaram *et al*., 1989; Verberne *et al*., 1990; Bazil & Gordon, 1993). To investigate the roles of mGluRs of IML in central autonomic activity, we performed experiments where agonists and antagonists selective for type I and II mGluRs have been administered intrathecally in conscious rats and the temporal changes in blood pressure and heart rate recorded. Our results provide the first evidence for spinal mGluRs being integrally involved in vasomotor function.

Methods

Surgery

Male Sprague-Dawly rats, weighing 300–350 g were anaesthetized with sodium methohexital (Brietal, Eli Lilly; 60 mg kg^{-1} i.p., supplemented as required), and underwent a two-stage operation. Initially, a polyethylene cannula (SP 8, ~5.5 cm long) was inserted through the atlanto-occipital membrane into the spinal subarachnoid space to lie at segments T_8-T_{10}, according to the method described previously (Yaksh & Rudy 1976; Verberne *et al*., 1990). At least 7 days later, a catheter was inserted into the right carotid artery for measurement of arterial blood pressure, from which mean arterial blood pressure (MAP) and heart rate (HR) were derived.

Experiments were begun in conscious rats 1–2 days after intravascular catheterization. Continuous recordings of blood pressure and heart rate were displayed on a MacLab-8 system (ADInstruments, Sydney, Australia) interfaced with a Macintosh computer.

Agonist studies

One group of rats was given intrathecal (i.t.) injections of Glu (1 μmol) and the mGluR agonist, *(1S,3R)*-ACPD (0.1 and 0.3 μmol) on separate days. Appropriate vehicles for each compound were also given at least 3 h before the agonist, or on a separate day. A second group of rats was treated in an identical manner, except that they received the selective type I mGluR agonist, (*RS*)-3,5-dihydroxyphenylglycine (DHPG; 0.03 and 0.1 μmol) and the type I/II mGluR agonist, (2*S*,3*S*,4*S*)-2-(carboxycyclopropyl)glycine (CCG-1; 0.1 and 0.3 μmol; Nicoletti *et al*., 1996) or vehicle. For both groups of rats, only one drug was tested per day and, where two doses were examined, at least 4 h was left between injections. A third group of rats received the type II mGluR agonist, 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (APDC), tested at two doses (0.1 and 1.0 μmol) on separate days.

Antagonist studies

From the results of the agonist studies, DHPG and APDC were chosen for further testing against the selective type I and II mGluR antagonists, (±)-1-aminoindan-1,5-dicarboxylic acid (AIDA) and (2*S*,4*S*)-2-amino-4(4,4-diphenylbut-1-yl)-pentane-1,5-doic acid (LY307452), respectively. One group of rats was given either AIDA (1 μmol i.t.) or vehicle followed 5 min later by DHPG (at a 10 fold lower dose than previously tested, 0.01 μmol i.t.) and the cardiovascular responses were recorded over 30 min. Another group of rats received LY307452 (0.5 μmol i.t.) or vehicle 5 min before APDC (1 μmol i.t.), was given, as described above. For both groups, vehicle and respective antagonists were given on different days and the order was randomized.

Drugs

Glutamate, APDC and DHPG were dissolved in artificial cerebrospinal fluid, while CCG-1, *(1S,3R)*-ACPD, AIDA and LY307452 were dissolved in 1 : 1 eq of NaOH and diluted in artificial cerebrospinal fluid as appropriate. Vehicle consisted of either artificial cerebrospinal fluid or an appropriate volume of 10 mM NaOH diluted in artificial cerebrospinal fluid. DHPG, CCG-1, *(1S,3R)*-ACPD, and AIDA were all purchased from Tocris (U.K.), while APDC and LY307452 were synthesized at Eli Lilly (Indianapolis, U.S.A.).

All drugs were given by i.t. injection in 10 μl volume flushed in with 5 μl aCSF over 0.5–1 min. Time course data presented are relative to the end of infusion period. At the completion of experiments, each rat was killed with an overdose of anaesthetic and the position of the intrathecal cannula was verified.

Statistical analysis

For agonist studies, the effects of all drugs and vehicles relative to their own predrug baseline were assessed by one-way ANOVA with repeated measures. For antagonist studies, a similar analysis was performed separately for antagonists (and vehicle) and agonists relative to their immediate baseline. In addition, the effect of AIDA/vehicle against DHPG and LY307452/vehicle against APDC were analysed by two-way ANOVA with repeated measures. Data from all cardiovascular variables are expressed as mean ± group standard error, which was calculated from the equation $\sqrt{}$EMS/n, where EMS is the error mean square from the ANOVA, and n is the number of rats in each group. A value of $P<0.05$ was taken as significant.

Results

Agonist studies

The i.t. injection of vehicle had negligible effect on MAP and HR. These effects, together with the cardiovascular effects of all mGluR agonists are summarized in Table 1. As expected, Glu (1 μmol, $n=6$) caused marked transient increases in MAP (max = 21 ± 3 mmHg, $P<0.01$) and HR (max = 84 ± 21 beats min^{-1}, $P<0.01$) lasting 5 and 20 min, respectively (Table 1). *(1S,3R)*-ACPD (0.1 μmol, $n=5$) evoked similar pressor and tachycardic responses as glutamate. A higher dose of *(1S,3R)*-ACPD (0.3 μmol, $n=3$) also increased MAP and HR to a similar extent such that no dose-related effect of *(1S,3R)*-ACPD was observed (Table 1).

The type I mGluR agonist DHPG (0.01 – 0.1 μmol) produced marked increases in MAP and HR. The duration of action, but not the maximum effect, appeared to be dose-related (Figure 1a,b; Table 1). Interestingly, approximately 5 min after DHPG (0.1 μmol) administration, rats started to exhibit particular behavioural effects such as forepaw licking, washing face together with gnawing and chewing. These behaviours were of a shorter duration than the cardiovascular effects, lasting for approximately 10 – 12 min, and appeared to be dose-related since these behavioural effects were more obvious with the highest dose of DHPG. The type I/II agonist CCG-1 (0.1 and 0.3 μmol) caused similar variable increases in MAP and HR, which were of a longer duration with the higher dose (Table 1). CCG-1 (0.3 μmol only) also evoked a behavioural response which was distinct from DHPG,

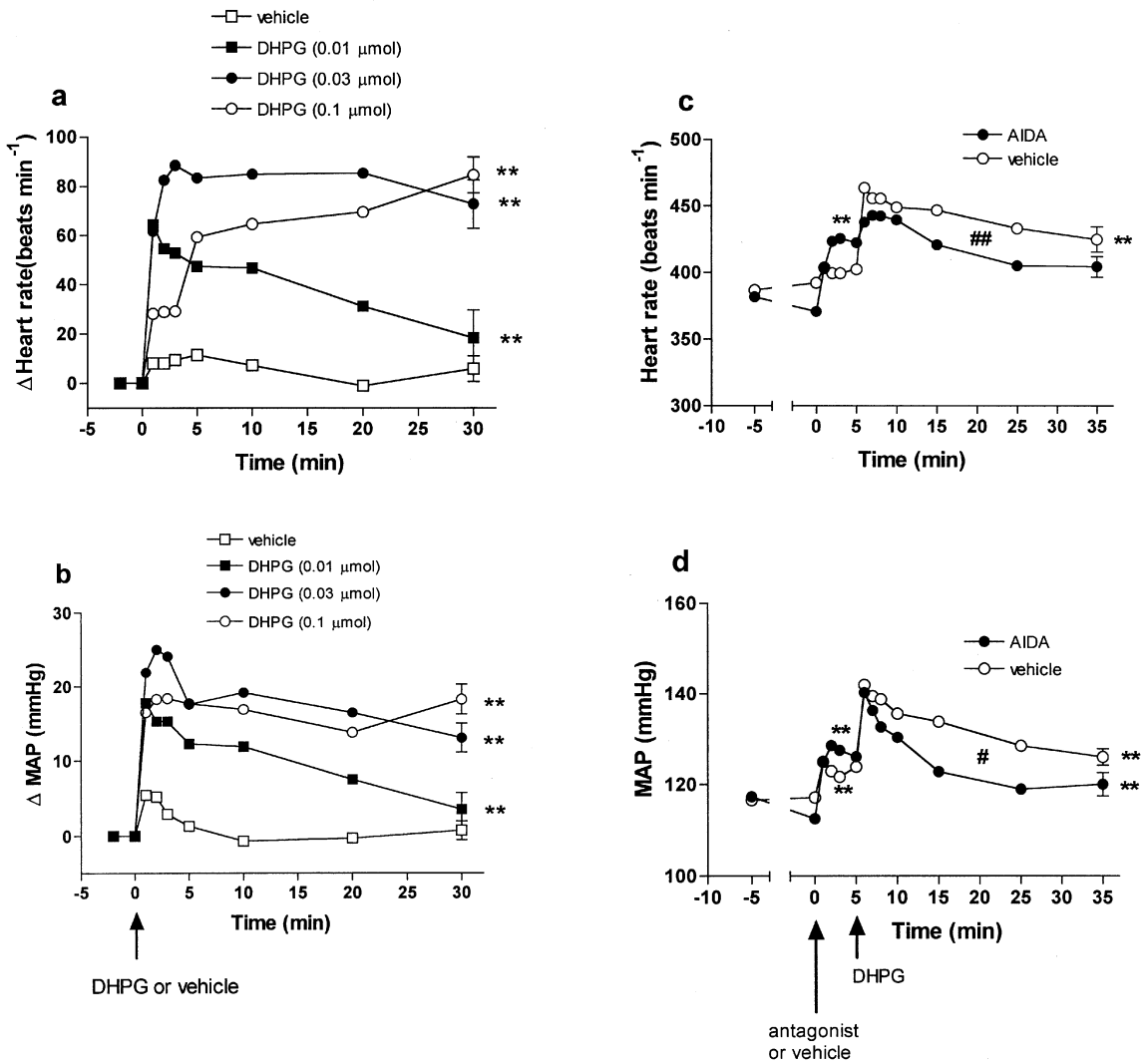

Figure 1 Time courses for changes in (a, c) heart rate (HR) and (b, d) mean arterial pressure (MAP) following i.t. injections of (a, b) the type I mGluR agonist DHPG (0.01 – 0.1 μmol, $n=3-5$) alone, or (c, d) DHPG (0.01 μmol i.t.) given 5 min after i.t. injection of vehicle ($n=5$) or the type I mGluR antagonist AIDA (1 μmol, $n=6$) in conscious rats. $^{**}P<0.01$ versus immediate pre-injection baseline for entire agonist/antagonist response (one-way ANOVA); $\#P<0.05$, $\#\#P<0.01$ for effect of treatment (vehicle/AIDA) on overall time course effect of DHPG (two-way ANOVA).

Table 1 Maximum changes in MAP and HR and duration of effect following i.t. injection of mGluR compounds

Drug	MAP			HR		
	Max Δ MAP (mmHg)	Time of Max (min)	Duration (min)	Max Δ HR (mmHg)	Time of Max (min)	Duration (min)
L-Glu						
1 μmol ($n=6$)	21±3**	1	5	84±21**	2	20
vehicle ($n=6$)	4±2	1	–	15±11	–	–
(1S,3R)-ACPD						
0.1 μmol ($n=5$)	18±1**	3	20	58±8**	10	20
0.3 μmol ($n=3$	18±1**	3	10	64±31	–	–
vehicle ($n=5$)	7±2*	1	1	12±4	–	–
DHPG						
0.01 μmol ($n=5$)	18±5**	1	10	64±14**	1	20
0.03 μmol ($n=3$)	25±5**	2	<60	88±23**	3	<60
0.1 μmol ($n=4$)	18±4**	3	<90	85±4**	30	>90
vehicle ($n=4$)	5±1	1	–	11±10	–	–
CGC						
0.1 μmol ($n=4$)	10±4**	1	5	54±10**	5	5
0.3 μmol ($n=4$)	12±2**	3	20	52±18	–	–
vehicle ($n=3$)	7±6	2	–	27±13	–	–
APDC						
0.1 μmol ($n=9$)	8±3	1	–	29±12*	3	3
1 μmol ($n=8$)	26±2**	1	5	59±13**	2	3

*$P<0.05$, **$P<0.01$ versus baseline.

consisting of grooming, rearing, head jerks and vocalization which began approximately 8–10 min after administration of CCG-1 and lasted for about 15 min.

The type II mGluR agonist APDC (0.1 μmol, $n=9$), produced minimal change in MAP and HR, while a 10 fold higher dose (1.0 μmol, $n=8$) caused marked, but transient, increases in MAP and HR (Figure 2a,b; Table 1). Overt behaviours were not observed subsequent to the administration of APDC.

Type I antagonist

DHPG (0.01 μmol, $n=5$) evoked a marked rise in MAP and HR which lasted for approximately 10 and 20 min, respectively. The prior administration of vehicle did not significantly alter HR and produced only a small pressor response (max = 8±1 mmHg, $P<0.01$) immediately before DHPG was given (Figure 1c,d). By contrast, AIDA (1 μmol, $n=6$) caused pressor (max = 16±6 mmHg, $P<0.01$) and tachycardic (max = 55±18 beats min^{-1}, $P<0.01$) responses, however, absolute MAP and HR values were not significantly different from the effects of vehicle immediately (i.e. at 5 min) prior to the administration of DHPG (Figure 1c,d). The subsequent injection of DHPG caused a significant pressor response (max = 14±4 mmHg, $P<0.01$), which was similar to that prior to vehicle administration, but no tachycardia. However, two-way ANOVA on the overall time course of changes in MAP and HR evoked by DHPG revealed that AIDA caused significant attenuation of both pressor ($P<0.05$) and tachycardic ($P<0.01$) effects compared with vehicle pretreatment (Figure 1c,d).

Type II antagonist

Vehicle caused negligible effects on MAP and HR prior to the administration of APDC. Thereafter, APDC (1 μmol, $n=8$) evoked significant increases in MAP and HR over 10 min (Figure 2c,d). By contrast, LY307452 (0.5 μmol, $n=6$). increased MAP (max = 18±4 mmHg, $P<0.01$) and HR (max = 38±7 beats min^{-1}, $P<0.01$) such that the absolute MAP and HR values were significantly different from the effects of vehicle immediately prior to the administration of APDC (Figure 2c,d). The subsequent injection of APDC did not cause a significant change in MAP and HR from pre-drug levels (Figure 2c,d). Two-way ANOVA on the overall time course of changes in MAP and HR evoked by APDC following LY307452 revealed a significant attenuation of the effect on MAP compared with vehicle pretreatment ($P<0.05$), while the difference between LY307452 and vehicle treatments was on the borderline of significance for HR ($P=0.07$).

Discussion

Two subtypes of Glu receptors, ionotropic and metabotropic, have been pharmacologically characterized, and have been shown to mediate the cardiovascular responses to Glu and other non-selective agonists when microinjected into various brain regions (see Introduction). Whilst the involvement of iGluRs in central cardiovascular regulation has been well studied, the role of mGluRs is less well understood. Given that the IML represents a major site for regulation of sympathetic nerve activity, we examined the cardiovascular responses to i.t. administration of mGluR agonists and antagonists in chronically instrumented conscious rats. Our results show that stimulation of type I or II mGluRs caused increased blood pressure and heart rate, providing evidence that both mGluR subtypes in the IML are involved in modulation of vasomotor function.

In the present study, Glu elicited marked, transient pressor and tachycardic responses, as has been reported previously using anaesthetized rats (Hong & Henry, 1992a,b). In addition, *(1S,3R)*-ACPD evoked a similar effect which contrasts with a recent study reporting no effect of this compound when injected into the spinal cord of anaesthetized rats (Arnolda *et al*., 1996). Three other selective mGluR agonists were tested and all increased MAP and HR, although their durations of action were noticeably different, the rank order being DHPG (type I) > CCG-1 (type I/II) > APDC (type II). These data support the view that both type I and II mGluRs participate in cardiovascular responses at the spinal level, but that type I mGluR activation exerts a greater influence under the present conditions. In this context, type I and II mGluRs

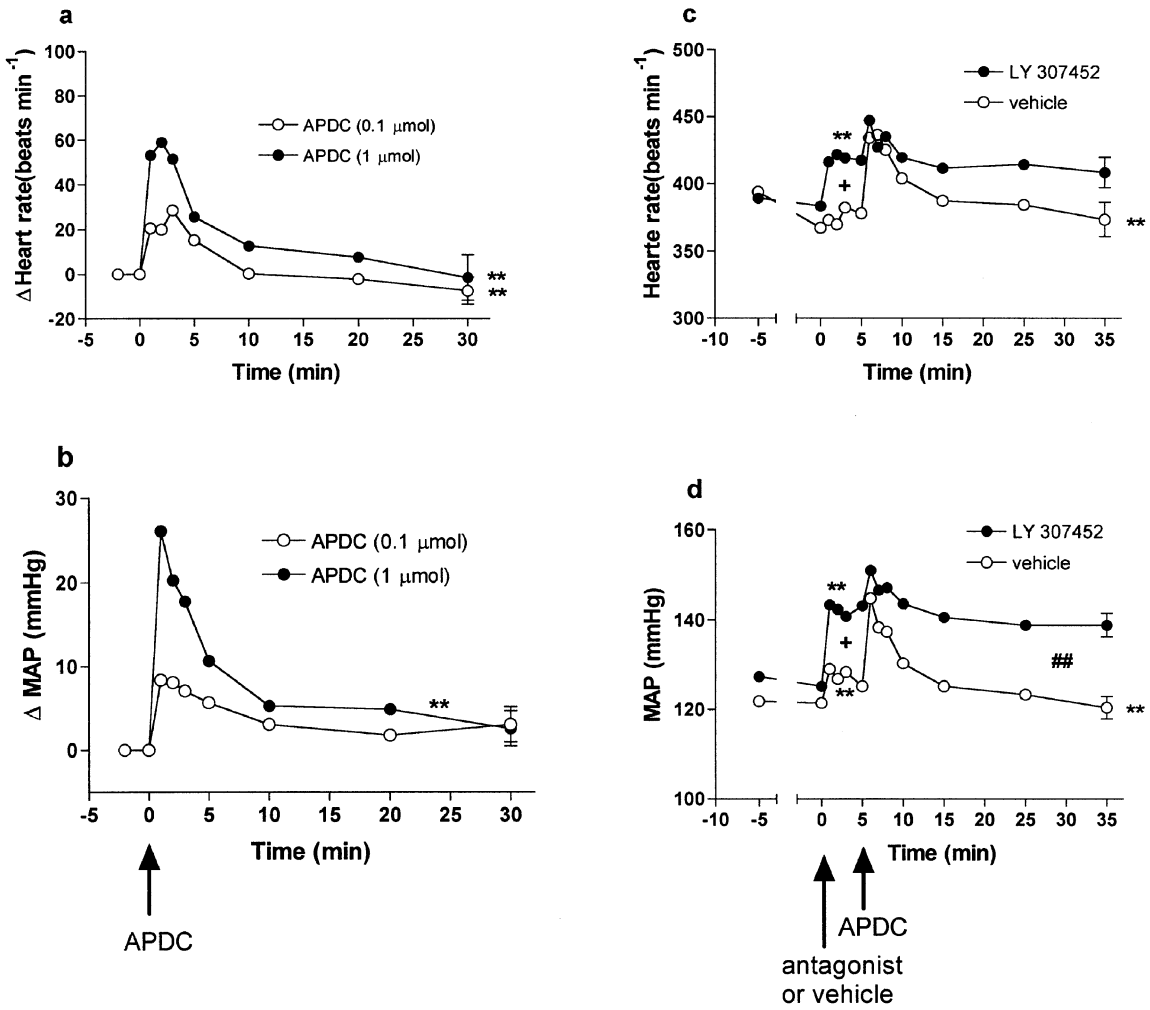

Figure 2 Time courses for changes in (a, c) heart rate (HR) and (b, d) mean arterial pressure (MAP) following i.t. injections of (a, b) the type II mGluR agonist APDC (0.1 μmol, $n=9$, and 1 μmol, $n=8$) alone, or (c, d) APDC (1 μmol i.t.) given 5 min after i.t. injection of vehicle ($n=6$) or the type II mGluR antagonist LY307452 (0.5 μmol, $n=6$) in conscious rats. $^{**}P<0.01$ versus immediate pre-injection baseline for entire agonist/antagonist response (one-way ANOVA); $+P<0.05$ for effect of treatment (vehicle/LY307452) (two-way ANOVA); $\#P<0.05$, $\#\#P<0.01$ for effect of treatment (vehicle/LY307452) on overall time course effect of APDC (two-way ANOVA).

in spinal cord slices have recently been described. Type I and II mGluRs mediated excitation and inhibition, respectively, of sympathetic preganglionic neurones, although the type I mGluR effect was the predominant one (Nolan & Logan, 1998). Conceivably, in the present *in vivo* setting, type I-mediated excitation may represent normal synaptic transmission at the spinal level, but an additional type II-mediated inhibitory action of APDC and, to a lesser extent, CCG-1 curtailed the initial excitatory effect.

In addition, the involvement of type I and II mGluRs in the IML of spinal cord in response to DHPG and APDC was evaluated using their selective antagonists, AIDA and LY307452, respectively. Unexpectedly, both AIDA and LY307452 alone also caused pressor and tachycardic responses, the reason for which is not immediately obvious given the antagonist profiles of these compounds documented in previous studies (Pelliacciari *et al*., 1995; Wermuth *et al*., 1996). One explanation might be that these subtype selective mGluRs antagonists exhibit partial agonistic activity, although this finding has not previously been noted (Pelliacciari *et al*., 1995; Wermuth *et al*., 1996). In any case, the effect of AIDA was short-lived such that baseline MAP and HR values prior to the injection of DHPG were similar in AIDA- and vehicle-treated rats. As expected, AIDA markedly attenuated the overall cardiovascular effects of DHPG, although the maximum increase in MAP was largely unaffected. Similarly, the type II mGLURs antagonist, LY307452, blunted the cardiovascular effects of APDC, although this intervention was performed at a time when LY307452 itself has raised baseline values.

Interestingly, the i.t. effects of the mGluR antagonists described here are in direct contrast to the effects of the iGluR antagonist, kynurenate, which caused a dramatic reduction in MAP and HR in conscious rats (Verberne *et al*., 1990), most likely by inhibition of sympathetic nerve discharge. These opposing actions of iGluR and mGluR antagonists are consistent with a growing body of evidence for contrasting actions of iGluR and mGluR agonists and antagonists (Leyva *et al*., 1995; D'Amico *et al*., 1996; Conn & Pin, 1997). In the present study, these effects are presumed to be highly localized since it has previously been shown that the small volumes injected i.t. are confined to the injection site T_8-T_{10} (Yaksh & Rudy, 1976; Verberne *et al*., 1990). Moreover, the cardiovascular effects are site-specific since we have recently shown that while AIDA and LY307452 exert no effects when injected into the NTS of rats, they attenuated the cardiovascular effects of

DHPG and APDC, respectively, at this central nucleus (Jones *et al*., 1999).

An interesting observation was that i.t. administration of the type I mGLURs agonist, DHPG and the type I/II mGLURs agonist, CCG-1, caused certain distinct behaviours effects such as forepaw licking, grooming and face washing, rearing, head jeck, and vocalization. These effects are very similar to a behavioural sequelae, termed spontaneous nociceptive behaviour, which has recently been reported (Fisher & Coderre, 1996). Consistent with the present study, type II mGluR agonists did not cause spontaneous nociceptive behaviour, which infers a type I mGluR-mediated response (Fisher & Coderre, 1996). This effect is unlikely to have contributed substantially to the cardiovascular changes noted since the cardiovascular actions persisted for much longer than the behavioural events.

In summary, in the present study we showed that i.t. administration of the mGluR agonists, DHPG (type I), CCG-1 (type I/II) and APDC (type II) markedly increased mean arterial blood pressure and heart rate. Prior i.t. administration of the type I and type II mGluRs antagonists, AIDA and LY307452, attenuated the cardiovascular responses to DHPG and APDC. Our results therefore suggest that functional type I and II mGluRs exist in the spinal cord, and that their activation may play an important role in the regulation of sympathetic outflow and thus cardiovascular homeostasis.

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