British Journal of Pharmacology (1997) 120, 1575–1581

The involvement of pertussis toxin-sensitive G proteins in the post receptor mechanism of central I_1-imidazoline receptors

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1 To elucidate the possible involvement of pertussis toxin (PTX)-sensitive G proteins in the post receptor mechanism of α_2-adrenoceptors and imidazoline receptors, we examined the effect of pretreatment of the central nervous system with PTX on the antidysrhythmic effect of dexmedetomidine, a selective α_2-adrenoceptor agonist, and rilmenidine, a selective I_1-imidazoline receptor agonist on halothane-adrenaline dysrhythmias in rats.

2 Dexmedetomidine (0, 1.0, 2.0, 5.0 μg kg^{-1} min^{-1}, i.v.) and rilmenidine (0, 1.0, 3.0, 10, 20 μg kg^{-1}, i.v.) prevented the genesis of halothane-adrenaline dysrhythmias in a dose-dependent fashion. Both idazoxan (10, 20 μg kg^{-1}, intracerebroventricularly (i.c.v.)), an α_2-adrenoceptor antagonist with high affinity for imidazoline receptors, and rauwolscine, (40 μg kg^{-1}, i.c.v.), an α_2-adrenoceptor antagonist with low affinity for imidazoline receptors inhibited the action of dexmedetomidine (5.0 μg kg^{-1} min^{-1}, i.v.), but the inhibitory potency of idazoxan was much greater than that of rauwolscine. While the pretreatment with PTX (0.1, 0.5, 1.0 μg kg^{-1}, i.c.v.) did not change the dysrhythmogenecity of adrenaline, this treatment completely blocked the antidysrhythmic property of rilmenidine (20 μg kg^{-1}, i.v.) as well as dexmedetomidine (5.0 μg kg^{-1} min^{-1}, i.v.).

3 It is suggested that central I_1-imidazoline receptors as well as α_2-adrenoceptors may be functionally coupled to PTX-sensitive G proteins.

Keywords: Anaesthetics, volatile, halothane; G protein, pertussis toxin; heart dysrhythmias; α_2-adrenoceptor; imidazoline receptors; sympathetic nervous system; catecholamines; adrenaline; α_2-agonist; dexmedetomidine; rilmenidine; α_2-antagonist; idazoxan; rauwolscine

Introduction

Guanine nucleotide binding proteins (G protein) are pivotally involved in the signal transduction of all adrenoceptor-mediated responses (Gilman, 1987). Bacterial toxins from *Bordetella Pertussis* can covalently modify one class of G proteins by the addition of an ADP-ribose group to a subunit. These pertussis toxin (PTX)-sensitive G proteins, which occupy 1–2% of the total membrane protein in mammalian brain (Sternweis *et al*., 1984), are well known to be involved in the signal transduction of α_2-adrenoceptor systems, including hypnosis and analgesia (Doze *et al*., 1990; Hayashi *et al*., 1995). We previously found that dexmedetomidine, a highly selective α_2-agonist (Savola *et al*., 1986), prevents the halothane-adrenaline-induced dysrhythmias via the central nervous system in dogs (Hayashi *et al*., 1991). Accordingly, the antidysrhythmic action of dexmedetomidine might be transduced via PTX-sensitive G proteins. On the other hand, recent investigations have demonstrated that some α_2-adrenoceptor ligands with an imidazoline structure or its derivatives, such as clonidine, beside being able to bind to α_2-adrenoceptors, also functionally bind to imidazoline receptors (IRs) (Bousquet *et al*., 1984; Lehmann *et al*., 1989; Wikberg *et al*., 1990; Tibirica *et al*., 1991a). Since dexmedetomidine has an affinity for IRs (Wikberg *et al*., 1990), we subsequently found the involvement of IRs as well as α_2-adrenoceptors in this modulation of the genesis of halothane-adrenaline dysrhythmias (Hayashi *et al*., 1993; Kamibayashi *et al*., 1995b). In contrast to α_2-adrenoceptors, the transmembrane signalling of IRs has not been well established and the involvement of G proteins in this mechanism is controversial. A recent binding study by Brica *et al*. (1994) refuted the coupling of IRs to G proteins, whereas a binding study by another group has claimed that IRs are also coupled to G proteins (Molderings *et al*., 1993).

The present study was designed to elucidate the role of PTX-sensitive G proteins in the post receptor mechanism of central α_2-adrenoceptors and IRs by use of the adrenaline-induced dysrhythmias model in halothane-anaesthetized rats. At first we confirmed the antidysrhythmic effect of dexmedetomidine and rilmenidine, a selective IR agonist (Vos *et al*., 1994), on halothane-adrenaline dysrhythmias in rats and defined the receptor mechanism of the antidysrhythmic action of dexmedetomidine. Then, to explore the involvement of PTX-sensitive G-protein in the signal transduction of IR- and α_2-adrenoceptor-mediated antidysrhythmic effect, we examined the effect of central pretreatment with PTX on the antidysrhythmic action of rilmenidine and dexmedetomidine.

Methods

Experimental preparation

This study was conducted under the guidelines approved by the Animal Care Committee of Osaka University, Faculty of Medicine. Two hundred and forty four Sprague Dawley male rats, weighing 350–450 g, were used and were housed in groups of four on a 12 h:12 h light-dark cycle with food and water *ad libitum*. The animals were anaesthetized with 1.5% halothane alone in 100% oxygen. After tracheotomy, the lungs were mechanically ventilated with a tidal volume of 4–5 ml at

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40–60 breaths min^{-1} (METRAN COMPOS β-EA, Tokyo, Japan). The respiratory rates were adjusted to maintain $PaCO_2$ at 35–45 mmHg. Inspired concentration of halothane was maintained at 1.5% (DATEX CAPNOMAC multiple gas monitor, Helsinki, Finland). Lead II of the electrocardiogram was monitored continuously. Catheters were inserted into a carotid artery for pressure monitoring and blood sampling, into a subclavian vein for injection of adrenaline, and into a femoral vein for administration of drugs. Arterial blood pressure was measured with a pressure transducer (Nihon Kohden AP-641G, Tokyo, Japan). Heart rate was counted by heart rate monitoring unit (Nihon Kohden AT-601G, Tokyo, Japan). The electrocardiogram and arterial blood pressure were recorded continuously with a thermal array recorder (Nihon Kohden WS-641G, Tokyo, Japan). A heating lamp was used to maintain rectal temperature at 37.5–38.5°C. Arterial pH and oxygen tension were maintained at 7.35–7.45 and more than 100 mmHg, respectively. After completion of preparation, anaesthesia was maintained for 30 min to achieve a steady state. Basal haemodynamic data (arterial blood pressure and heart rate) were then measured.

Determination of dysrhythmogenic dose of adrenaline

The dysrhythmogenic dose (DD) of adrenaline was defined as the smallest dose that produced three or more premature ventricular contractions within 15 s of injection. As we did previously, adrenaline was injected at logarithmically spaced doses (0.5, 1.0, 1.41, 2.0, 2.83, 4.0, 5.67, 8.0, etc. $\mu g\ kg^{-1}$) following an initial dose of 4.0 $\mu g/kg^{-1}$ (Takada *et al*., 1993). The 4.0 $\mu g\ kg^{-1}$ dose served as an indicator of the direction in which to proceed in order to establish the DD, i.e., higher or lower dose of adrenaline. This method could decrease the number of adrenaline injections necessary to determine DD. A period of 10–30 min was allowed between each injection until the haemodynamic parameters (arterial blood pressure and heart rate) became stable.

Determination of plasma adrenaline concentration

When the criterion for DD was satisfied, a 2 ml arterial blood sample was collected to allow measurement of the plasma concentration (PC) of adrenaline. The blood samples were put into pre-cooled plastic tubes containing 20 μl 0.2 M EDTA-2Na and 0.2 M $Na_2S_2O_5$, which were then centrifuged at 4,000 r.p.m. for 10 min at 2°C to separate the plasma. For analysis of adrenaline, 0.5 ml plasma was acidified by the addition of 0.25 ml 2.5% perchloric acid to precipitate protein. The samples were stored at −40°C for not longer than 7 days, until analysis. The plasma concentration of adrenaline was determined in a fully automated high-performance liquid chromatography-fluorometric system (model HLC-8030 Catecholamine Analyzer, Tosoh, Tokyo, Japan), by a diphenylethylendiamine condensation method (Nohta *et al*., 1984). This assay method has a limit of sensitivity of 10 pg ml^{-1} for adrenaline. The inter- and intra-assay variations were less than 3%.

Drugs and administration

Dexmedetomidine and rilmenidine were kind gifts from Farmos Pharmaceutica (Turku, Finland) and Institut de Recherches Servier (France), respectively. Other chemicals were obtained from the sources indicated: halothane (Takeda Chemical, Osaka, Japan), PTX (Sigma Chemical, MO, U.S.A.), idazoxan hydrochloride (RBI, MA, U.S.A.), rauwolscine hydrochloride (RBI), (−)-adrenaline (Wako chemical, Osaka, Japan). Dexmedetomidine was dissolved in saline at a concentration of 100 $\mu g\ ml^{-1}$. Rilmenidine was dissolved in saline to the desired concentration such that each rat received a dose as a 0.2 ml bolus injection. Idazoxan and rauwolscine were dissolved in saline and PTX was dissolved in phosphate buffer to the desired concentration such that each rat received a dose in a volume of 10 μl. In each rat, the cerebroventricle was stereotactically cannulated with a 30 G stainless steel needle according to the following co-ordinates: with the bregma as the reference, 1.5 mm lateral, 0.8mm posterior, and at a depth of 3.5mm from the skull. PTX and antagonists were administered intracerebroventricularly through the needle. Adrenaline was dissolved in 0.1 ml HCl (1 N) and diluted with saline to the desired concentration such that each rat received a dose as a 0.1 ml bolus injection.

Experimental protocols

Experiment 1: The effect of dexmedetomidine and rilmenidine on the halothane-adrenaline-induced dysrhythmias ($n=78$) We determined the DD and PC of adrenaline in the presence of dexmedetomidine 0, 1.0, 2.0, 5.0 $\mu g\ kg^{-1}\ min^{-1}$, or rilmenidine 0, 1.0, 3.0, 10 and 20 $\mu g\ kg^{-1}$. Dexmedetomidine, rilmenidine or vehicle was administered intravenously and then 30 min later the first injection of adrenaline was started.

Experiment 2: Antagonistic activity of idazoxan and rauwolscine on the effect of dexmedetomidine ($n=69$) In order to confirm the receptor mechanism involved in the action of dexmedetomidine, the DD and PC of adrenaline were examined in the presence of dexmedetomidine (5.0 $\mu g\ kg^{-1}\ min^{-1}$) combined with either idazoxan, an α_2-adrenoceptor antagonist with high affinity for imidazoline receptors (IRs), or rauwolscine, an α_2-adrenoceptor antagonist with low affinity for IRs. Since previous data documented that idazoxan exerts similar α_2-adrenoceptor antagonistic potency to rauwolscine (Shepperson *et al*., 1981; Boyajian *et al*., 1987; Parini *et al*., 1989; Feldman *et al*., 1990; Illes & Norenberg, 1990; Tibirica *et al*., 1991a), the doses of each antagonist were determined so that they produced roughly equieffective α_2-adrenoceptor antagonistic potency, that is, the dose range tested for idazoxan and rauwolscine was 1.0, 10 and 20 $\mu g\ kg^{-1}$, and 2.0, 20 and 40 $\mu g\ kg^{-1}$, respectively. These antagonists or vehicle were administered intracerebroventricularly in a volume 10 μl, 15 min before the start of the dexmedetomidine infusion and then 30 min later the first injection of adrenaline was started.

Experiment 3: The effect of PTX on the antidysrhythmic action of dexmedetomidine and rilmenidine ($n=97$) We studied the effect of pretreatment with intracerebroventricular PTX on the antidysrhythmic action of dexmedetomidine or rilmenidine. Various doses of PTX (0.1, 0.5, and 1.0 μg) or vehicle in a volume of 10 μl were administered via the intracerebroventricular route under halothane anaesthesia. We determined the DD and PC of adrenaline during halothane anaesthesia in the presence of dexmedetomidine 5.0 $\mu g\ kg^{-1}\ min^{-1}$, rilmenidine 20 $\mu g\ kg^{-1}$, or vehicle on day 4 after the administration of PTX or vehicle, because a previous study had documented that it took three days for PTX to be ribosylated and for PTX-sensitive G proteins to become inactivated (Aghanjanian & Wang, 1986). Dexmedetomidine, rilmenidine or vehicle was administered intravenously and then 30 min later the first injection of adrenaline was started. In each experiment, haemodynamic data (arterial blood pressure and heart rate) were recorded at the time the DD was achieved under the different experimental conditions.

Data analysis

The data are expressed as means ± s.e.mean. Statistical significance of data was analysed by one-way analysis of variance, and comparisons between groups were assessed by Scheffe's test. We considered $P<0.05$ to be statistically significant.

Results

Dexmedetomidine significantly increased the DD and the plasma concentration of adrenaline required to induce dys-

rhythmias in a dose-dependent manner (Figure 1). Rilmenidine, a selective IR agonist, also exerted a dose-dependent antidysrhythmic action on halothane-adrenaline dysrhythmias (Figure 2). Although both idazoxan and rauwolscine inhibited the antidysrhythmic action of dexmedetomidine, the inhibition by idazoxan was greater than that of rauwolscine when the two antagonists were compared at doses possessing roughly equipotent α_2-adrenoceptor antagonistic activity (Figure 3). Pretreatment with PTX did not affect the genesis of halothane-adrenaline dysrhythmias in the absence of dexmedetomidine and rilmenidine (Figure 4), but did produce a dose-dependent attenuation of the antidysrhythmic effect of dexmedetomidine, achieving almost complete inhibition of the effect of dexmedetomidine at 0.5 μg (Figure 5). The antidysrhythmic effect of rilmenidine was similarly inhibited by the PTX pretreatment (Figure 6). The haemodynamic data at the onset of dysrhythmias in the presence of dexmedetomidine and rilmenidine are shown in Table 1 and 2, respectively. Although both the systolic and diastolic arterial pressures increased in the presence of both drugs, the only significant changes were those for diastolic pressure at the highest dose of dexmedetomidine (Table 1) and for systolic pressure with the highest dose of rilmenidine (Table 2). In comparison, neither idazoxan nor rauwolscine modified the haemodynamic parameters at the onset of dysrhythmias in the dexmedetomidine-treated animals

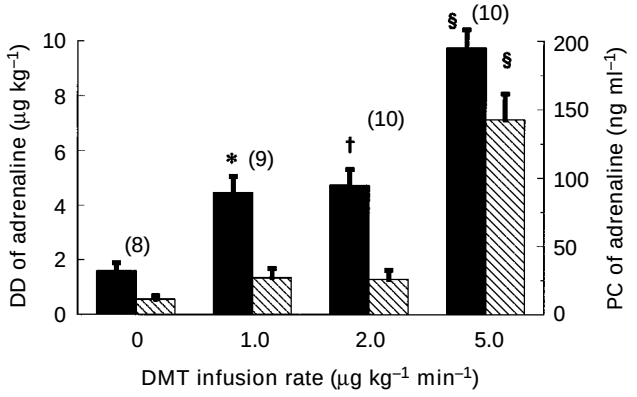


Figure 1 The dysrhythmogenic threshold of adrenaline in the presence of dexmedetomidine (DMT) 0, 1.0, 2.0, and 5.0 μg kg^{-1} min^{-1} during halothane anaesthesia (mean$\pm$s.e.mean of number of observations shown in parentheses). *$P<0.05$ compared with the 0 dose. †$P<0.01$ compared with the 0 dose. §$P<0.01$ compared with the 0, 1.0, 2.0 dose. Solid columns, dysrhythmogenic dose (DD); hatched columns, plasma concentration (PC).

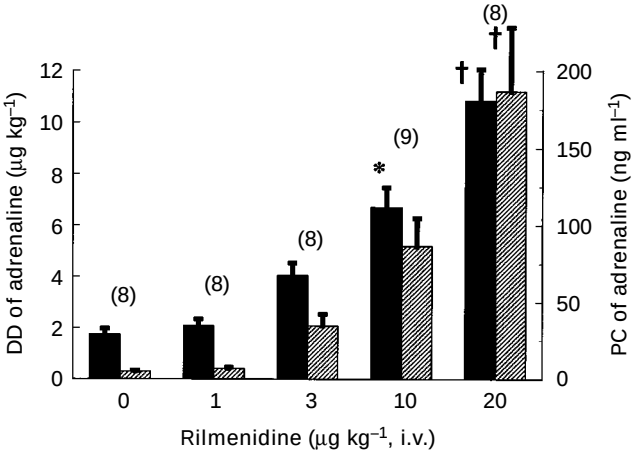
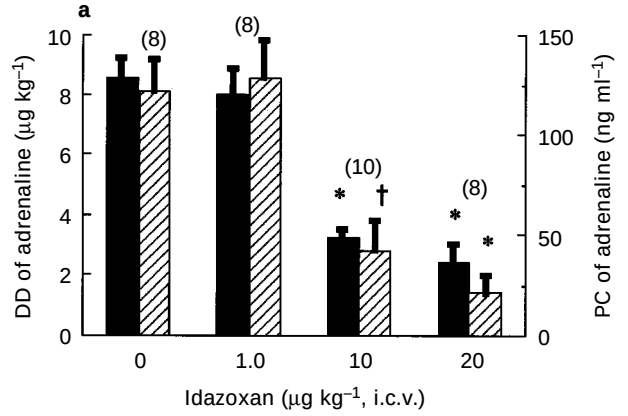


Figure 2 The dysrhythmogenic threshold of adrenaline in the presence of rilmenidine 0, 1.0, 3.0, 10 and 20 μg kg^{-1} during halothane anaesthesia (mean$\pm$s.e.mean of number of observations shown in parentheses). *$P<0.01$ compared with the 0, 1.0 dose. †$P<0.01$ compared with the 0, 1.0, 3.0 dose. Solid columns, dysrhythmogenic dose (DD); hatched columns, plasma concentration (PC).



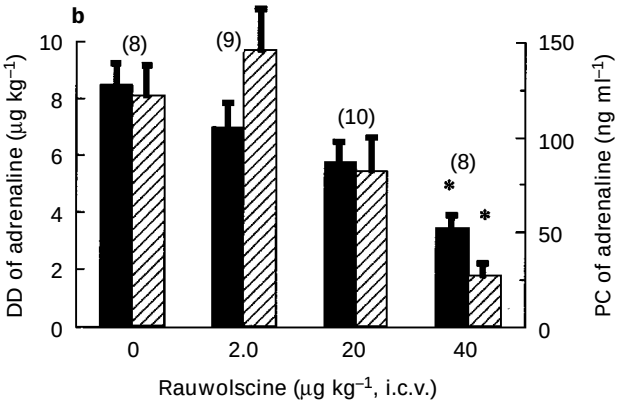


Figure 3 The effect of α_2-adrenoceptor antagonists on the dysrhythmogenic threshold of adrenaline in the presence of dexmedetomidine 5.0 μg kg^{-1} min^{-1} during halothane anaesthesia (mean$\pm$s.e.mean of number of observations shown in parentheses). (a) The effect of idazoxan 0, 1.0, 10, 20 μg kg^{-1} given intracerebroventricularly (i.c.v.). *$P<0.01$ compared with the 0, 1.0 dose. †$P<0.05$ compared with the 0, 1.0 dose. (b) The effect of rauwolscine 0, 2.0, 20, 40 μg kg^{-1} given i.c.v. *$P<0.01$ compared with the 0, 2.0 dose. Solid columns, dysrhythmogenic dose (DD); hatched columns, plasma concentration (PC).

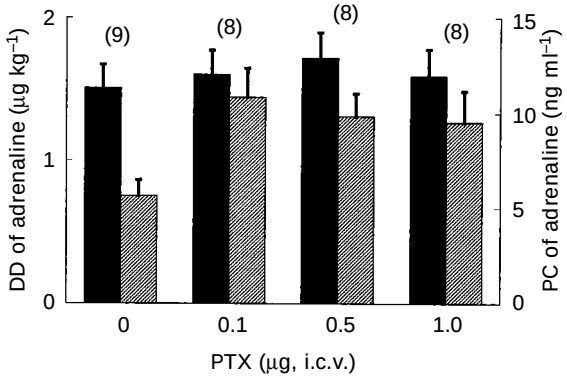


Figure 4 The effect of pertussis toxin (PTX) given intracerebroventricularly (i.c.v.) on the dysrhythmogenic threshold of adrenaline during halothane anaesthesia (mean$\pm$s.e.mean of number of observations shown in parentheses). Solid columns, dysrhythmogenic dose (DD); hatched columns, plasma concentration (PC).

(Table 3). The haemodynamic data for the combination of PTX with dexmedetomidine or rilmenidine is shown in Table 4. PTX 1.0 μg significantly decreased the systolic and diastolic arterial pressures at the onset of dysrhythmias in the presence of dexmedetomidine. In comparison, there were no significant haemodynamic changes during the dysrhythmias in the rilmenidine- and PTX-treated animals.

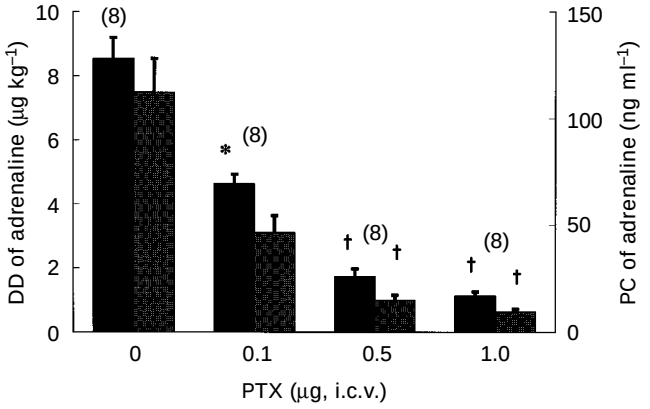


Figure 5 The effect of pertussis toxin (PTX) given i.c.v., on the dysrhythmogenic threshold of adrenaline in the presence of dexmedetomidine 5.0 μg kg^{-1} min^{-1} during halothane anaesthesia (mean $\pm$ s.e.mean of number of observations shown in parentheses). *$P<0.01$ compared with the 0 dose. †$P<0.01$ compared with the 0, 0.1 dose. Solid columns, dysrhythmogenic dose (DD); stippled columns, plasma concentration (PC).

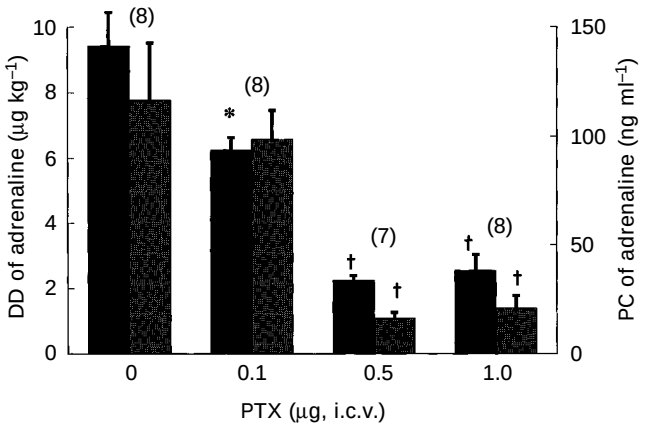


Figure 6 The effect of pertussis toxin (PTX) given i.c.v., on the dysrhythmogenic threshold of adrenaline in the presence of rilmenidine 20 μg kg^{-1} during halothane anaesthesia (mean $\pm$ s.e.mean of number of observations shown in parentheses). *$P<0.01$ compared with the 0 dose. †$P<0.01$ compared with the 0, 0.1 dose. Solid columns, dysrhythmogenic dose (DD); stippled columns, plasma concentration (PC).

Discussion

Receptor mechanism of the antidysrhythmic action of dexmedetomidine and rilmenidine

The significant role of the imidazoline receptor (IR) in the physiological regulation of the central nervous system was advocated by Bousquet *et al*. (Bousquet *et al*., 1984; Tibirica *et al*., 1991a), who demonstrated that the IR is involved in the hypotensive effect of clonidine which was traditionally believed to be exerted through activation of α_2-adrenoceptors. Later, the IR has been documented to be responsible for several other pharmacological properties of α_2-adrenoceptor agonists (Potter & Ogidigben, 1991; Maiese *et al*., 1992). There is also evidence indicating that this receptor is distinct from the α_2-adrenoceptor (Boyajian & Leslie, 1987; Parini *et al*., 1989; Kamisaki *et al*., 1990). Although dexmedetomidine is a selective α_2-adrenoceptor agonist (Savola *et al*., 1986), it has an affinity for the IR (Wikberg & Uhlen, 1990) and our previous study with dogs demonstrated that IRs were more responsible for the antidysrhythmic action of dexmedetomidine than α_2-adrenoceptors (Kamibayashi *et al*., 1995b). The present study in rats also shows that idazoxan which possesses an affinity for IRs exerts a more potent inhibition of the antidysrhythmic effect of dexmedetomidine than rauwolscine which has low affinity for IRs when the two antagonists were compared at doses possessing roughly equipotent α_2-adrenoceptor antagonistic activity. Therefore, IRs as well as α_2-adrenoceptors are involved in the antidysrhythmic action of dexmedetomidine in our dysrhythmia model. Furthermore, it has now become established that IRs have two subtypes, namely I_1 and I_2 (Michel & Ernsberger, 1992; Ernsberger *et al*., 1993). Rilmenidine, which is a selective I_1 receptor agonist with low affinity for the I_2 receptor (Ernsberger *et al*., 1993; Renouard *et al*., 1993), exerts an antidysrhythmic effect exclusively through IRs (Mammoto *et al*., 1995), significantly increased the dysrhythmogenic threshold of adrenaline in a dose-dependent manner (Figure 2). Collectively, these findings confirm the significant involvement of I_1 receptors in the modulation of halothane-adrenaline dysrhythmias. The present results cannot identify the precise site in the central nervous system involved in the antidysrhythmic action of dexmedetomidine and rilmenidine.

Table 2 Haemodynamic data at the onset of dysrhythmias in the presence of rilmenidine during halothane anaesthesia

Dose of Rilmenidine (μg kg^{-1})	n	*SAP* (mmHg)	*DAP* (mmHg)	*HR* (beats min^{-1})
0	8	153 $\pm$ 8.0	112 $\pm$ 5.8	380 $\pm$ 9.9
1.0	8	164 $\pm$ 6.5	115 $\pm$ 3.9	379 $\pm$ 14
3.0	8	166 $\pm$ 4.8	120 $\pm$ 3.6	376 $\pm$ 8.2
10.0	9	175 $\pm$ 4.3	130 $\pm$ 3.7	377 $\pm$ 8.0
20.0	8	185 $\pm$ 4.0*	131 $\pm$ 3.8	370 $\pm$ 8.8

SAP = systolic arterial pressure; DAP = diastolic arterial pressure; HR = heart rate. Values are mean $\pm$ s.e.mean. *$P<0.01$ compared with rilmenidine 0 value.

Table 1 Haemodynamic data at the onset of dysrhythmias in the presence of dexamedetomidine during halothane anaesthesia

Dose of dexamedetomidine (μg kg^{-1} min^{-1})	n	*SAP* (mmHg)	*DAP* (mmHg)	*HR* (beats min^{-1})
0	8	150 $\pm$ 6.6	110 $\pm$ 6.1	370 $\pm$ 7.5
1.0	9	153 $\pm$ 5.4	115 $\pm$ 5.1	347 $\pm$ 8.2
2.0	10	169 $\pm$ 4.7	126 $\pm$ 3.0	366 $\pm$ 7.3
5.0	10	170 $\pm$ 3.3	133 $\pm$ 4.5*	372 $\pm$ 5.2

SAP = systolic arterial pressure; DAP = diastolic arterial pressure; HR = heart rate. Values are mean $\pm$ s.e.mean. *$P<0.05$ compared with dexamedetomidine 0 value.

Table 3 The effect of α_2-adrenoceptor antagonists on haemodynamic data at the onset of dysrhythmias in the presence of dexmedetomidine (5.0 μg kg^{-1} min^{-1}) during halothane anaesthesia

Dose of idazoxan (μg kg^{-1})	n	*SAP (mmHg)*	*DAP (mmHg)*	*HR* (*beats min*$^{-1}$)
0	8	178±2.3	141±2.6	371±6.5
1.0	8	189±4.5	135±6.9	370±9.3
10	10	174±2.7	131±5.9	386±10
20	8	169±4.3	138±5.2	379±5.5
Dose of rauwolscine (μg kg^{-1})				
0	8	171±4.1	136±4.1	374±6.0
2.0	9	174±2.7	134±2.9	380±4.0
20	10	171±4.0	131±4.4	385±4.6
40	8	179±3.6	127±3.8	387±10

SAP = systolic arterial pressure; DAP = diastolic arterial pressure; HR = heart rate. Values are mean±s.e.mean.

Table 4 The effect of pertussis toxin (PTX) on haemodynamic data at the onset of dysrhythmias in the presence of (A) dexmedetomidine (5.0 μg kg^{-1} min^{-1}) or (B) rilmenidine (20.0 μg kg^{-1}) during halothane anaesthesia

Dose of PTX (μg kg^{-1})	n	*SAP (mmHg)*	*DAP (mmHg)*	*HR* (*beats min*$^{-1}$)
(A)				
0	8	178±2.3	141±2.6	371±6.4
0.1	8	170±3.3	128±2.2	387±5.0
0.5	8	174±7.4	133±5.5	380±11
1.0	8	154±4.7*	119±3.6*	363±5.3
(B)				
0	8	187±3.9	131±7.6	362±9.0
0.1	8	178±3.5	128±4.2	373±10
0.5	7	164±4.7	109±2.6	369±9.3
1.0	8	174±8.7	121±7.6	373±4.7

SAP = systolic arterial pressure; DAP = diastolic arterial pressure; HR = heart rate. Values are mean±s.e.mean. *$P<0.05$ compared with PTX 0 value.

I_1 receptors are located predominantly in the rostral ventrolateral medulla oblongata (RVLM) of the brainstem (Bricca *et al*., 1994), whereas I_2 receptors are located in several other brain nuclei particularly in the cerebral cortex (Reis *et al*., 1992). I_1 receptors in the C1 area of the RVLM have been shown to be responsible for the hypotensive action of clonidine (Ernsberger *et al*., 1987; Tibirica *et al*., 1991a,b) and rilmenidine (Gomez *et al*., 1991). Accordingly, the C1 area of the RVLM might be one possible region in which the antidysrhythmic action originates. This brain area is connected functionally with the nucleus tractus solitarius (NTS) which modulates autonomic control, including vagal activity (Ross *et al*., 1985), which plays a critical role in the antidysrhythmic property of dexmedetomidine and rilmenidine (Kamibayashi *et al*., 1995a; Mammoto *et al*., 1995).

Involvement of PTX-sensitive G proteins in the antidysrhythmic action of dexmedetomidine and rilmenidine

PTX contains a ribosylase that catalyses the attachment of adenosine diphosphate-ribose to a conserved cystine residue four amino acids from the carboxyl terminus of the α-subunit of PTX-sensitive G proteins (Hoshino *et al*., 1990). Once G proteins are ribosylated by PTX, they cannot dissociate following the activation of the receptor by its agonist. Thus, pharmacological effects of the agonist are attenuated (Ui, 1984; Gilman, 1987). With this strategy, PTX has been frequently used to examine the involvement of PTX-sensitive G proteins in several biological and pharmacological phenomena. Although myocardial sensitization to the dysrhythmogenic effects of adrenaline is one of the typical pharmacological effects of halothane (Atlee & Bosnijak, 1990), the present results show that intracerebroventricular pretreatment with PTX did not change the dysrhythmogenic threshold of adrenaline (Figure 4), indicating that PTX-sensitive G proteins in the central nervous system do not mediate the myocardial sensitization to adrenaline by halothane.

On the other hand, the same PTX treatment attenuated the antidysrhythmic effects of dexmedetomidine in a dose-dependent manner and almost abolished the effects of the medium PTX dose we tested (Figure 5). Taken together with the receptor mechanism involved in the antidysrhythmic action of dexmedetomidine mentioned above, this result may indicate that PTX-sensitive G proteins are involved in the transduction of the antidysrhythmic action of dexmedetomidine which is mediated through I_1 receptors as well as α_2-adrenoceptors. Furthermore, the antidysrhythmic action of rilmenidine, which is a selective I_1 receptor agonist with low affinity for I_2 receptors (Ernsberger *et al*., 1993; Renouard *et al*., 1993), was also completely blocked by PTX (Figure 6). These results suggest that not only α_2-adrenoceptors but also I_1 receptors in the central nervous system are coupled with PTX-sensitive-G proteins. It is well known that PTX-sensitive G proteins are involved in the signal transduction of several α_2-adrenoceptor systems. For example, hypnosis, analgesia, and depression of the firing of locus coeruleus neurones have all been demonstrated to be transduced via PTX-sensitive G proteins (Aghanjanian & Wang, 1986; Doze *et al*., 1990; Hayashi *et al*., 1995). Accordingly, the antidysrhythmic response to dexmedetomidine mediated by α_2-adrenoceptors was expected to be inhibited by pretreatment with PTX. In comparison, somewhat surprisingly, PTX-sensitive G proteins were also involved in the antidysrhythmic action mediated via I_1 receptors. Although two previous studies have demonstrated that the signal transduction of IRs is different from that of α_2 adrenoceptors (Parini *et al*., 1989; Regunathan *et al*., 1991), two recent radioligand binding studies came to the opposite conclusion. Brica *et al*. (1994) documented that IRs in the ventrolateral medulla of the human brainstem were not coupled to G proteins, while Molderings *et al*. (1993) showed that IRs in bovine adrenal chromaffin cells were G protein coupled receptors. The present functional study supports the latter opinion and a recent biological study has suggested that there is a strong link between I_2 receptors and monoamine oxidase (Tesson *et al*., 1995). Accordingly, it is possible that the discrepant results of the two binding studies are due to separate subtypes of IRs, the I_1 receptor being coupled to PTX-sensitive G proteins and involved in the antidysrhythmic property of the agents we tested. Furthermore, Ernsberger *et al*. (1995) have suggested that I_1 receptors are modulated by guanine nucleotides with a specificity appropriate for a receptor coupled to G proteins and activation of phospholipase A_2 plays a major role in the signalling pathway of the I_1 receptors.

Haemodynamic parameters and dysrhythmias

Haemodynamic parameters have been shown to be an important factor in modulating the genesis of halothane-adrenaline dysrhythmias (Reynolds, 1984; Atlee & Bosnijak, 1990). Although dexmedetomidine and rilmenidine increased the dysrhythmogenic threshold of adrenaline in a dose-dependent manner, cardiovascular changes associated with these changes of dysrhythmogenic threshold were not so marked (Table 1 and 2). An α_2-adrenoceptor agonist is well known to enhance the baroreflex response to an increase in blood pressure (Harron *et al*., 1985), and a recent study has documented that rilmenidine also possesses this property (Spiers *et al*., 1990). Accordingly, dexmedetomidine and rilmenidine may attenuate the elevation of blood pressure following the adrenaline infusion and this effect might contribute to the antidysrhythmic action of these drugs.

We conclude that I_1 receptors as well as α_2-adrenoceptors in the central nervous system play a significant role in the antidysrhythmic effect of dexmedetomidine and rilmenidine. Although PTX-sensitive G proteins are not involved in the myocardial sensitization by halothane to the dysrhythmogenic action of adrenaline, the antidysrhythmic action of dexmedetomidine and rilmenidine are mediated by this group of proteins, indicating that I_1 receptors are coupled to PTX-sensitive G proteins.

The authors wish to thank Institut de Recherches Internationales Servier (France) and Farmos Pharmaceutica (Turku, Finland) for supplying rilmenidine and dexmedetomidine, respectively. They also appreciate Ms K. Akama, Ms T. Uemura and Ms A. Ohta for their assistance throughout this study. This manuscript is supported by a grant from the Japanese Cardiovascular Research Foundation.

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(Received September 23, 1996
Revised January 2, 1997
Accepted January 22, 1997)