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Prejunctional α_2-adrenoceptors in mouse atria function through G-proteins which are sensitive to N-ethylmaleimide, but not pertussis toxin

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1 The identity of the G-proteins involved in prejunctional α_2-adrenoceptor signal transduction in mouse atria was examined by use of the G-protein inactivators N-ethylmaleimide and pertussis toxin.

2 The α_2-adrenoceptor partial agonist clonidine (0.03 μM) inhibited the electrical stimulation-induced (S-I) outflow of radioactivity from mouse atria which were incubated with [^{3}H]-noradenaline and stimulated at 5 Hz. The partial α_2-adrenoceptor agonist St 363 (10 μM) inhibited the S-I outflow of radioactivity at the lower stimulation frequency of 2.5 Hz. The inhibitory effects of these compounds were not altered in mice pretreated with pertussis toxin (1.5 μg, i.v.).

3 The α_2-adrenoceptor antagonist, idazoxan (0.1 μM), increased the S-I outflow of radioactivity from mouse atria stimulated at 5 Hz, and this effect was not altered in atria from mice pretreated with pertussis toxin.

4 The inhibitory effects of clonidine and St 363 and the facilitatory effect of idazoxan on the S-I outflow of radioactivity from mouse atria were significantly less in atria incubated with N-ethylmaleimide (NEM, 3 μM) for 60 min before the [^{3}H]-noradrenaline incubation.

5 The results suggest that prejunctional α_2-adrenoceptors in mouse atria function through G-proteins which are NEM-sensitive, but pertussis toxin insensitive.

Keywords: G-proteins; prejunctional α_2-adrenoceptors; noradrenaline release; N-ethylmaleimide; pertussis toxin

Introduction

Structurally, α_2-adrenoceptors belong to the guanosine 5'-triphosphate (GTP)-binding protein (or G-protein) coupled family of receptors (Zeng *et al.*, 1990; Harrison *et al.*, 1991). The effects of α_2-adrenoceptor-stimulation are blocked by pertussis toxin, an agent which inactivates some G-proteins (G_i and G_o) by adenosine diphosphate (ADP)-ribosylation (see Ui, 1988). These effects include lipidolysis in fat cells (García-Sáinz, 1981), inhibition of insulin release from rat pancreatic islets (Katada & Ui, 1981) and vasoconstriction in the pithed rat (Boyer *et al.*, 1983; Docherty, 1988; Nichols *et al.*, 1988).

Activation of α_2-adrenoceptors results in inhibition of noradrenaline release from sympathetic nerves (see Starke, 1977; 1987). However, Musgrave *et al.* (1987) demonstrated that prejunctional α_2-adrenoceptors on noradrenergic nerves in mouse atria were not sensitive to pertussis toxin. In addition, pertussis toxin did not alter the inhibitory effect of the α_2-adrenoceptor agonists on cardiac responses to sympathetic nerve stimulation in the pithed rat, even though responses to postjunctional α_2-adrenoceptors in the same studies were attenuated by pertussis toxin (Docherty, 1988; Nichols *et al.*, 1988). Pertussis toxin treatment also did not affect prejunctional α_2-adrenoceptors in rat vas deferens (Docherty, 1988; 1990; however see Lai *et al.*, 1983), mouse vas deferens (Lux & Schultz, 1986), rat atria (Docherty, 1990), rat tail artery (Li, 1990) or rabbit renal artery (Wolk *et al.*, 1991). Furthermore in rat kidney, pertussis toxin abolished the inhibitory effect of the α_1-adrenoceptor agonist, methoxamine but had no effect on the inhibition produced by clonidine (Murphy & Majewski, 1990). These results are in contrast to studies performed on brain tissue, where pertussis toxin attenuated the inhibitory effects of α_2-adrenoceptor agonists on [^{3}H]-noradrenaline release from rabbit hippocampus (Allgaier *et al.*, 1985).

The aim of the present study was to use N-ethylmaleimide (NEM) in mouse atria to determine further whether G-proteins were involved in α_2-adrenoceptor signal transduction in sympathetic nerves. NEM alkylates proteins and is capable of inactivating G-proteins with some degree of selectivity over other cellular structures (Winslow *et al.*, 1987; Ueda *et al.*, 1990). NEM also alkylates G-proteins associated with α_2-adrenoceptors (Kitamura & Nomura, 1987), and has been shown to attenuate α_2-adrenoceptor-mediated inhibition of noradrenaline release from rat tail artery (Weber, 1989; Illes *et al.*, 1990), mouse vas deferens (Kaschube & Brasch, 1990) and rabbit renal artery (Wolk *et al.*, 1991). In contrast to previous studies we decided to use St 363 as our test α_2-adrenoceptor agonist as it is a weak partial agonist on prejunctional α_2-adrenoceptors (Medgett & McCulloch, 1980) and thus must activate a larger proportion of α_2-adrenoceptors to exert its effect which may make it easier to detect changes in functional α_2-adrenoceptors.

Methods

Pertussis toxin treatment

Female random bred Swiss white mice (17–38 g) were treated with pertussis toxin (1.5 μg/mouse in 0.1 ml lysine-glycerol buffer, i.v.) or vehicle solution (0.1 ml lysine-glycerol buffer, i.v.), four days before the experiment.

Atria were taken from vehicle- and pertussis toxin-treated mice. The mice were stunned, decapitated and the whole hearts removed and placed in pre-warmed (37°C) Krebs-Henseleit solution which was continuously gassed with a

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mixture of 95% O_2/5% CO_2. The atria were dissected free and suspended in an organ bath containing 2.5 ml Krebs-Henseleit solution maintained at 37°C and continuously gassed with a mixture of 95% O_2/5% CO_2. The basal tension applied to the atria was 0.1 g. The negative chronotropic effects of carbachol (10 μM) on the spontaneously beating whole atria were determined. The atria were equilibrated for 1 h in Krebs-Henseleit solution.

The atria were then incubated with (−)-[7,8-^{3}H]-noradrenaline (0.2 μM, 2.9 μCi ml^{-1}) for 20 min. After this incubation period, the atria were washed every 2 min for 60 min with fresh Krebs-Henseleit solution. After 45 min of this washing procedure an electrical priming stimulation (5 Hz, 60 s, square wave pulses of 15 V cm^{-1}, 1 ms duration) was delivered through two platinum field electrodes. Following the washing period, the bathing solution was collected every 3 min for 54 min. There were two periods of electrical stimulation of the atria, at 9 and 39 min after the start of the sampling period, termed S_1 and S_2 respectively. Drugs were added to the bathing solution from 15 min before the second stimulation period.

At the end of the sampling period the atria were dissolved in 2 ml Soluene (Packard Instruments, U.S.A.). The amount of radioactivity in these samples and the superfusate samples was determined by adding 6 ml Picofluor-40 (Packard Instruments, U.S.A.) to each sample followed by liquid scintillation counting. Corrections were made for counting efficiency with an automatic external standard and results were expressed as disintegrations per minute (d.p.m.).

Calculation of results

The resting (spontaneous) outflow of radioactivity from the atria was taken as the mean radioactive content of the superfusate during the 3 min period immediately before stimulation and the 3 min period starting 6 min after the onset of stimulation. The stimulation-induced (S-I) outflow of radioactivity was calculated by subtracting the total amount of radioactivity in the calculated spontaneous outflow from the total amount of radioactivity in the two 3 min samples collected during and immediately after stimulation.

In all cases the S-I outflow of radioactivity was expressed as a fraction of the total tissue content of radioactivity at the time of stimulation (fractional S-I outflow). The fractional S-I outflow of radioactivity in the second stimulation period (S_2) is expressed as a percentage of that in the first (S_1). The corresponding spontaneous outflows of radioactivity are referred to as R_2 and R_1.

Incubation of mouse atria with N-ethylmaleimide

To allow direct comparison with the pertussis toxin experiments all mice were treated with lysine-glycerol buffer. Some atria from vehicle-treated mice were incubated with N-ethylmaleimide (NEM). After dissection, atria were incubated in drug-free Krebs-Henseleit solution or Krebs-Henseleit solution containing NEM (3 μM) for 60 min. The incubation solution was replaced with fresh solution every 10 min. At the end of this incubation period the atria were washed with drug-free Krebs-Henseleit solution every 1 min for 5 min and the negative chronotropic effects of carbachol (10 μM) on the spontaneously beating whole atria were determined. Experiments on [^{3}H]-noradrenaline outflow from these atria were then carried out.

Statistics

Data are expressed as means ± the standard error of the mean (s.e.mean). Statistical analysis of the data was performed by one- or two-way analyses of variance (ANOVA) followed by unpaired *t* tests between individual groups of data, unless otherwise specified. Where multiple means were compared to a single control value, a Dunnett's test was performed to compare the data. Values of $P < 0.05$ were taken to indicate significant differences between means. The statistical tests were performed with the computer programme CSS (Statsoft, Tulsa, U.S.A.).

Drugs and vehicles

The composition of the Krebs-Henseleit solution was as follows (all concentrations in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_3 1.3, $MgSO_4$ 0.45, D-(+)-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.14.

Pertussis toxin was obtained from Commonwealth Serum Laboratories, Parkville, Victoria. The pertussis toxin was dissolved in 50 mM Tris-HCl buffer/lysine glycerol solution (pH 8.3).

The composition of the lysine-glycerol buffer used to dissolve the pertussis toxin further was (all concentrations in mM): NaCl 250, $NaHCO_3$ 25 and L-lysine 10. All reagents were initially dissolved in deionized water and the pH adjusted to 7.5. Glycerol was then added in a 1:1 ratio to give the above concentrations.

The following drugs were purchased: (−)-[7,8-^{3}H]-noradrenaline (Radiochemical Centre, Amersham, U.K.; specific activity 10.6 Ci mmol^{-1}); carbamyl choline (carbachol), N-ethylmaleimide (Sigma Chemical Co., St. Louis, MO, U.S.A.).

The following drugs were generously donated: idazoxan (Reckitt and Colman, U.K.); clonidine hydrochloride and St 363 (2-(2,4-dichlorophenylimino)-imidazolidine; Boehringer Ingelheim, Australia). All drugs were initially dissolved in deionized water and subsequently diluted in Krebs-Henseleit solution.

Results

Effect of carbachol on spontaneous rate of beating of isolated atria

Carbachol (10 μM) completely inhibited the beating of atria from vehicle-treated mice (0.1 ml lysine-glycerol, i.v.). However, carbachol (10 μM) had no effect on the spontaneous beating of atria isolated from any pertussis toxin-treated mouse (1.5 μg/mouse, i.v.; Table 1).

Carbachol (10 μM) reduced, but did not abolish the spontaneous beating of mouse atria incubated with NEM (3 μM for 60 min) (Table 1). The basal rate of beating of atria incubated with NEM was significantly lower than that of vehicle incubated atria (Table 1).

Influence of pertussis toxin on the effects of clonidine, St 363 and idazoxan in mouse atria

The uptake of radioactivity into, and the resting and the S-I outflow of radioactivity from atria taken from pertussis toxin treated (1.5 μg, i.v.) mice and incubated in [^{3}H]-noradrenaline was not different from that in atria taken from vehicle-treated mice (Table 2).

At a stimulation frequency of 5 Hz, the S-I outflow of radioactivity from the atria of vehicle-treated mice was inhibited by clonidine (0.03 μM) and enhanced by idazoxan (0.1 μM; Figure 1) and the respective effects of clonidine or idazoxan were not significantly different in atria from pertussis toxin-treated mice (Figure 1). The partial α_2-adrenoceptor agonist St 363 (10 μM) had no effect on the S-I outflow of radioactivity from vehicle-treated mouse atria at a stimulation frequency of 5 Hz, but significantly reduced the S-I outflow of radioactivity at 2.5 Hz (Figure 1). The inhibitory effect of St 363 in atria taken from pertussis toxin-treated mice at 2.5 Hz was not significantly different from that observed in atria of vehicle-treated mice (Figure 1). Neither clonidine, St 363 nor idazoxan altered the resting outflow of radioactivity from the mouse atria (not shown).

Table 1 The influence of pertussis toxin or N-ethylmaleimide (NEM) on the effect of carbachol (10 μM) on the spontaneous rate of beating of mouse isolated atria

Treatment	n	*Rate of beating* (beats min^{-1}) *Before carbachol*		*After carbachol*
Mouse atria				
Vehicle	49	350 ± 11	---*---	0 ± 0
Pertussis toxin	23	346 ± 18		333 ± 22
NEM	37	320 ± 18	--*--	132 ± 24

Mice (1.5 μg, i.v.) were treated with pertussis toxin or vehicle solution 4 days before the experiment. NEM incubation (3 μM for 60 min) took place before incubation with [^{3}H]-noradrenaline.
*Indicates a significant effect of carbachol $P < 0.05$ (Student's t test). The inhibitory effect of carbachol was significantly reduced in NEM-incubated atria and atria from pertussis toxin-treated animals compared to that in atria from vehicle-treated animals ($P < 0.05$, two-way ANOVA). Data are represented as means ± s.e.mean. n = number of experiments in each group.

Table 2 Effect of pertussis toxin or N-ethylmaleimide (NEM) on outflow and uptake of [^{3}H]-noradrenaline from mouse atria

Parameter	*Stim. Freq.* (Hz)	*Vehicle*	n	*Treatment pertussis toxin*	n	*NEM*	n
S_1, %	5	1.49 ± 0.13	17	1.31 ± 0.07	15	0.71 ± 0.04▲	21
	2.5	0.63 ± 0.06*	24	0.68 ± 0.13*	8	0.48 ± 0.04*▲	16
	1.75	0.50 ± 0.04*	8				
R_1, %		0.98 ± 0.04	49	0.91 ± 0.04	23	1.49 ± 0.07▲	37
Tissue radioactivity (d.p.m. × 10^{-6})		1.06 ± 0.05	49	1.30 ± 0.13	23	0.71 ± 0.05▲	37

Mice were treated with vehicle solution (0.1 ml lysine-glycerol, i.v.) or pertussis toxin (1.5 μg, i.v.) 4 days before the experiment. NEM incubation (3 μM for 60 min) took place immediately before incubation with [^{3}H]-noradrenaline. There were two periods of electrical stimulation of the atria, S_1 and S_2, 30 min apart, at either 5, 2.5 or 1.75 Hz for 60 s. The fractional S-I outflow of radioactivity in S_1 (S_1, %), the fractional spontaneous outflow of radioactivity corresponding to this stimulation (R_1, %) and the total content of radioactivity at the start of the sampling period (d.p.m.) are shown. Data are represented as means ± s.e.mean. Significant differences from the S-I outflow of radioactivity at 5 Hz are represented by *, which indicates $P < 0.5$ (Student's t test after ANOVA). ▲Indicates a significant effect of NEM compared to the appropriate parameter in atria from vehicle-treated mice ($P < 0.05$, Student's t test after ANOVA).

Influence of NEM on the effects of clonidine and idazoxan in mouse atria

The uptake of radiaoctivity into mouse atria incubated with NEM before incubation with [^{3}H]-noradrenaline was significantly lower than the uptake into atria incubated in vehicle solution (Table 2). Incubation with NEM also reduced the S-I outflow of radioactivity compared with vehicle-incubated atria, at all frequencies tested (Table 2). In mouse atria incubated with NEM, the resting outflow of radioactivity was higher than that from atria incubated in vehicle solution (R_1; Table 2).

At a stimulation frequency of 5 Hz, clonidine (0.03 μM) had no effect on the S-I outflow of radioactivity from the mouse atria pre-incubated with NEM (3 μM for 60 min; Figure 2), in contrast to its inhibitory effect in vehicle-incubated atria. The enhancing effect of idazoxan (0.1 μM) on the S-I outflow of radioactivity at 5 Hz was significantly less in atria incubated with NEM than in atria incubated in vehicle solution (Figure 2). NEM also reduced the inhibitory effect of clonidine at a lower stimulation frequency of 2.5 Hz ($P < 0.05$, ANOVA; Figure 2).

Another comparison of the effects of clonidine was performed under equivalent absolute S-I outflow conditions (2.5 Hz/vehicle vs. 5 Hz/NEM; see Table 2). At 2.5 Hz, clonidine inhibited the S-I outflow of radioactivity from vehicle-treated atria to a significantly greater extent than at 5 Hz (Figure 2). When the effects of clonidine in vehicle and NEM pre-incubated atria were compared under conditions of equivalent S-I outflow of radioactivity (2.5 Hz/vehicle vs. 5 Hz/NEM), the inhibitory effect of clonidine in NEM pre-incubated atria (5 Hz stimulation) was markedly less than that observed in vehicle pre-incubated atria (2.5 Hz stimulation; $P < 0.05$, ANOVA; Figure 2).

The facilitatory effect of idazoxan on the S-I outflow of radioactivity in vehicle-treated atria was significantly less at 2.5 Hz than at 5 Hz (Figure 2). When the effects of idazoxan in vehicle and NEM pre-incubated atria were compared under conditions of equivalent S-I outflow of radioactivity (2.5 Hz/vehicle versus 5 Hz/NEM), the facilitatory effect of idazoxan in NEM pre-incubated atria (5 Hz stimulation) was still significantly less than that at 2.5 Hz in atria from vehicle-treated mice ($P < 0.05$, ANOVA; Figure 2). None of the drugs altered the resting outflow of radioactivity (not shown).

Influence of NEM on the effect of St 363 in mouse atria

St 363 significantly inhibited the S-I outflow of radioactivity from mouse atria incubated with [^{3}H]-noradrenaline and stimulated at 2.5 Hz (Figure 2). In atria pre-incubated with NEM (3 μM for 60 min) and stimulated at 2.5 Hz, St 363 (10 μM) had no effect on the S-I outflow of radioactivity (Figure 2). Another set of experiments was performed in the absence of NEM where the stimulation frequency was lowered to 1.75 Hz so that the fractional S-I outflow of radioactivity was not significantly different from the NEM-incubated atria at 2.5 Hz (Table 2). At 1.75 Hz, St 363 inhibited the S-I outflow of radioactivity from vehicle-treated atria to a significantly greater extent than at 2.5 Hz (Figure 2) and significantly greater extent than from NEM-treated atria at 2.5 Hz ($P < 0.05$, ANOVA; Figure 2). None of the drugs affected the resting outflow of radioactivity (not shown).

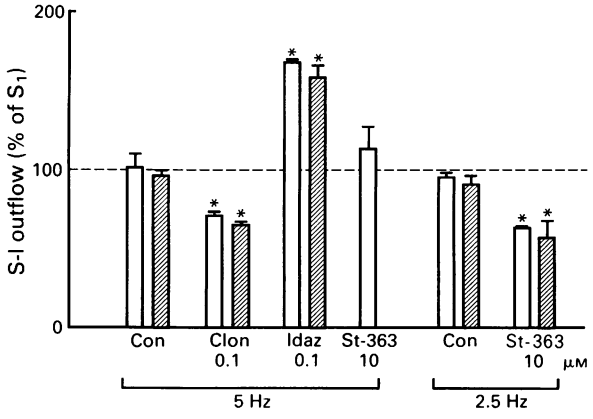

Figure 1 Influence of pertussis toxin treatment on the effects of clonidine (Clon, 0.1 μM), idazoxan (Idaz, 0.1 μM) and St 363 (10 μM) on the S-I outflow of radioactivity from mouse atria incubated in [^{3}H]-noradrenaline. Mice were treated with pertussis toxin or vehicle solution (i.v.) 4 days before the experiment. There were two periods of electrical stimulation of the atria, S_1 and S_2, 30 min apart, both 5 or 2.5 Hz for 60 s. Drugs were present from 15 min before the second stimulation period. The columns represent the fractional S-I outflow of radioactivity in S_2 as a percentage of that in S_1, and show the means with s.e.mean indicated by vertical bars. Significant differences from control experiments (Con) are represented by *, which indicates $P < 0.05$ (Student's t test, after ANOVA). The open columns refer to vehicle experiments and the hatched columns are atria from pertussis toxin-treated mice (1.5 μg). The inhibitory effects of clonidine and St 363 and the facilitatory effect of idazoxan in atria from pertussis toxin-treated mice were not significantly different from those in atria from vehicle-treated mice ($P > 0.05$, ANOVA). The number of experiments in each group (n) is as follows: for vehicle-treated mice, Con (5 Hz) = 7, Clon = 6, Idaz = 4, St 363 = 4, Con (2.5 Hz) = 11, St 363 = 4; for pertussis toxin-treated mice, Con (5 Hz) = 5, Clon = 6, Idaz = 4, Con (2.5 Hz) = 4, St 363 = 4.

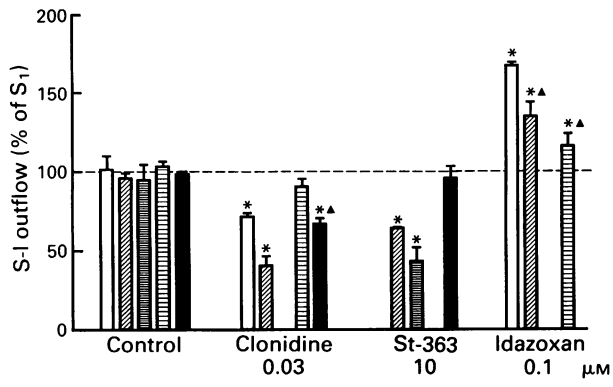

Figure 2 Influence of incubation with N-ethylmaleimide (NEM, 3 μM for 60 min) on the effects of clonidine (Clon, 0.1 μM), idazoxan (Idaz, 0.1 μM) or St 363 (10 μM) on the S-I outflow of radioactivity from mouse atria incubated in [^{3}H]-noradrenaline. There were two periods of electrical stimulation of the atria, S_1 and S_2, 30 min apart, 5, 2.5 or 1.75 Hz for 60 s. Clonidine, idazoxan or St 363 was present from 15 min prior to the second stimulation period. The columns represent the fractional S-I outflow of radioactivity in S_2 as a percentage of that in S_1, and show the means with s.e.mean indicated by vertical bars. The open columns refer to vehicle experiments (5 Hz), the oblique hatched columns are vehicle 2.5 Hz, the narrow spaced horizontal hatched are vehicle 1.75 Hz, the wide space horizontal hatched are NEM-treated 5 Hz and the black columns are NEM-treated 2.5 Hz. Significant differences from corresponding control experiments (Con are represented by *, which indicates $P < 0.05$ (Student's t test, after ANOVA). ▲Indicates that the effects of clonidine and idazoxan were significantly reduced in atria incubated with NEM from those in vehicle-incubated atria ($P < 0.05$, two-way ANOVA). The number of experiments in each group (n) is as follows: for atria from vehicle-treated mice (5 Hz), Con = 7, Clon = 6, Idaz = 4; (2.5 Hz) Con = 11, Clon = 4, St 363 = 4, Idaz = 5; (1.75 Hz) Con = 4, St 363 = 4; for NEM-incubated atria (5 Hz), Con = 9, Clon = 7, Idaz = 6; (2.5 Hz) Con = 5, Clon = 5, St 363 = 5, Idaz = 5.

Discussion

In the present study, clonidine inhibited and idazoxan enhanced stimulation-induced noradrenaline release from mouse atria which suggests that automodulatory, prejunctional α_2-adrenoceptors were functional. In atria from mice treated with the G-protein inactivator, pertussis toxin (Ui, 1988), the effects of clonidine and idazoxan on noradrenaline release were not different from those observed in atria from vehicle-treated mice. In addition, the pertussis toxin treatment had no effect on the absolute levels of noradrenaline release *per se*. All of these results are consistent with a lack of effect of pertussis toxin on the α_2-autoinhibitory mechanism and this is in agreement with an earlier study in mouse atria (Musgrave *et al.*, 1987).

One possible reason for these negative results is that pertussis toxin may have inactivated only a portion of the G-protein pool and if there were an excess of prejunctional α_2-adrenoceptors than that necessary for a full response (spare receptors) then this may not be sufficient to diminish the effects of α_2-adrenoceptor ligands. In rat hepatocytes at least 80% of the G-proteins had to be inactivated to reduce the inhibitory effect of angiotensin II on adenosine 3':5'-cyclic monophosphate (cyclic AMP) production (Pobiner *et al.*, 1985). We therefore used St 363 as an α_2-adrenoceptor agonist since it has a lower efficacy than clonidine (pD_2 of 5.5 compared to 8.8 for clonidine in guinea-pig atria; Medgett & McCulloch, 1980) and therefore has to activate a greater proportion of the α_2-adrenoceptors than clonidine to produce its effect. Indeed, at stimulation frequency of 5 Hz, St 363 had no effect on noradrenaline release (unlike clonidine) and an inhibitory effect was only revealed at a stimulation frequency of 2.5 Hz, presumably because only at the lower frequency was the concentration of neuronally-released noradrenaline low enough to enable St 363 to exert an effect. This frequency-dependence of the effects of partial α_2-adrenoceptor agonists has been shown previously (Medgett *et al.*, 1978; Rand *et al.*, 1980; Rump & Majewski, 1987). Pertussis toxin treatment had no influence on the inhibitory effect of St 363 on noradrenaline release from the mouse atria which again argues against a role for pertussis toxin-sensitive G-proteins in α_2-adrenoceptor inhibition of noradrenaline release. It should be noted that the pertussis toxin treatment was sufficient to inhibit completely muscarinic receptor-mediated bradycardia, an event mediated by pertussis toxin-sensitive G-proteins (e.g. Martin *et al.*, 1985). Similar negative results with pertussis toxin on α_2-adrenoceptor modulation of noradrenaline release have been obtained in other sympathetically innervated tissues (see Introduction). However, Nozaki & Sperelakis (1989) reported that pertussis toxin reduced the inhibitory effect of clonidine on noradrenaline release from the guinea-pig mesenteric artery.

Another tool to investigate G-proteins is N-ethylmaleimide (NEM), which alkylates sulphydryl groups. Despite this relatively non-selective action, with widespread effects at higher concentrations (see for other effects: Smith & Lauf, 1985; Schömig *et al.*, 1988; Sabatini & Kurtzman, 1989; Wölfel *et al.*, 1989; Foucart *et al.*, 1990), it has been suggested that NEM targets G-proteins selectively at lower concentrations (Winslow *et al.*, 1987; Ueda *et al.*, 1990). It has also been reported that pertussis toxin and NEM may inactivate the same G-proteins (Kitamura & Nomura, 1987; Ueda *et al.*, 1990). However, NEM may also affect G-proteins which are not pertussis toxin-sensitive. In mouse atria incubated with NEM, the inhibitory effect of neuropeptide Y and the facilitatory effects of angiotensin II and isoprenaline were reduced by NEM but not by pertussis toxin

(Foucart *et al.*, 1990). Importantly, NEM did not affect the isoprenaline or angiotensin II signal transduction pathways – cyclic AMP and protein kinase C respectively – suggesting that the NEM targeted receptor/G-protein interactions (Foucart *et al.*, 1990). Some caution must nevertheless be used since NEM by itself reduced [^{3}H]-noradrenaline release and the uptake of [^{3}H]-noradrenaline in mouse atria (Foucart *et al.*, 1990) and similar effects were seen in the present study. The reasons for this are unclear but suggest that either alkylation of G-proteins affect these processes or that the effects of NEM are not entirely selective.

In the present study the inhibitory effect of clonidine on S-I noradrenaline release was only slightly attenuated in mouse atria pre-incubated with NEM. One complicating factor in these results is that NEM by itself reduced the S-I outflow of noradrenaline from the mouse atria, as has been previously reported in mouse atria (Foucart *et al.*, 1990). Therefore it may be more appropriate to compare the effects of α_2-adrenoceptor ligands under conditions of equivalent S-I noradrenaline release, since it is well known that in the presence of a lower biophase concentration of noradrenaline, the inhibitory effect of exogenous partial α_2-adrenoceptor agonists like clonidine and St 363 are increased (present study; also Medgett *et al.*, 1978; Rand *et al.*, 1980). When compared under these conditions of equivalent S-I noradrenaline release (2.5 Hz vehicle versus 5 Hz NEM), the inhibitory effect of clonidine was clearly reduced by NEM. Similarly, under these conditions of equivalent noradrenaline release (1.75 Hz vehicle versus 2.5 Hz NEM), the inhibitory effect of St 363 was also clearly reduced in NEM-incubated atria. The facilitatory effect of idazoxan on noradrenaline release from NEM-incubated atria was significantly attenuated, but not abolished, when compared with that in vehicle-treated atria. This effect may have been entirely attributable to the reduction of noradrenaline release caused by NEM, which would result in less 'feedback' inhibition of noradrenaline release. However, when the effects of idazoxan were examined under conditions of equivalent noradrenaline release (2.5 Hz vehicle versus 5 Hz NEM), the facilitatory effect of idazoxan was still significantly attenuated in NEM-incubated atria when compared to that in vehicle-treated atria. This is an indication that the feedback inhibitions exerted by equivalent amounts of neuronally-released noradrenaline is inhibited by NEM. Taken together, these results obtained with clonidine, St 363 and idazoxan suggest that prejunctional α_2-adrenoceptors in mouse atria function through NEM-sensitive G-proteins. Similar effects of NEM on prejunctional α_2-adrenoceptors have been observed in other tissues (see Introduction).

The results suggest that prejunctional α_2-adrenoceptors in mouse atria function through G-proteins which are sensitive to NEM, but not to pertussis toxin, as also observed in rabbit renal artery (Wolk *et al.*, 1991). The question then arises as to which G-protein is involved in prejunctional α_2-adrenoceptor transduction in these tissues. Previous studies have suggested that NEM and pertussis toxin inactivate the G-proteins, G_i and G_o, primarily by acting at the same site, as NEM treatment prevents ADP-ribosylation of these G-proteins by pertussis toxin (Winslow *et al.*, 1987; Hertting & Allgaier, 1988; Ueda *et al.*, 1990). It is possible that NEM affected some other site on the G-protein α-subunit rather than the ADP-ribosylation site, as NEM alkylates several sulphydryl groups in the α-subunit that appear to be functionally important (Asano & Ogasawara, 1986; Winslow *et al.*, 1987); however, this still does not explain the ineffectiveness of pertussis toxin. The most likely explanation seems to be that prejunctional α_2-adrenoceptors in mouse atria function through G-proteins that are sensitive to NEM but not pertussis toxin. Previous results suggest that G-proteins other than G_i and G_o are affected by NEM (Sigafoos & Bramowitz, 1986; Foucart *et al.*, 1990). However, the exact nature of the G-protein involved in prejunctional α_2-adrenoceptors signal transduction on sympathetic nerve varicosities remains an enigma.

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