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# Assessment of the role of $\alpha_2$-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice

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**1** The role of $\alpha_2$-adrenoceptor (AR) subtypes in the modulation of acute nociception, motor behaviour and body temperature, has been investigated by determining the activity of the $\alpha_2$AR selective agonist dexmedetomidine (Dex) in mice devoid of individual $\alpha_2$AR subtypes through either a point ($\alpha_{2A}$) or null ($\alpha_{2B}/\alpha_{2C}$) mutation ('knock-out').

**2** In a rodent model of acute thermal nociception, the mouse tail immersion test, Dex, in wild type (WT) control animals, produced a dose-dependent increase in the threshold for tail withdrawal from a 52°C water bath with mean $ED_{50}$ values of $99.9 \pm 14.5$ ($\alpha_{2A}$), $94.6 \pm 17.8$ ($\alpha_{2B}$) and $116.0 \pm 17.1$ ($\alpha_{2C}$) $\mu g\ kg^{-1}$, i.p.

**3** In comparison to the WT controls, Dex (100–1000 $\mu g\ kg^{-1}$, i.p.), was completely ineffective as an antinociceptive agent in the tail immersion test in the $\alpha_{2A}$AR D79N mutant animals. Conversely, in the $\alpha_{2B}$AR and $\alpha_{2C}$AR knock-outs, Dex produced a dose-dependent antinociceptive effect that was not significantly different from that observed in WT controls, with $ED_{50}$ values of $85.9 \pm 15.0$ ($P > 0.05$ vs WT control) and $226.0 \pm 62.7$ ($P > 0.05$ vs WT control) $\mu g\ kg^{-1}$ i.p., respectively.

**4** Dex (10–300 $\mu g\ kg^{-1}$, i.p.) produced a dose-dependent reduction in spontaneous locomotor activity in the $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$AR WT control animals with $ED_{50}$ values of $30.1 \pm 9.0$, $23.5 \pm 7.1$ and $32.3 \pm 4.6$ $\mu g\ kg^{-1}$, i.p., respectively. Again, Dex (100–1000 $\mu g\ kg^{-1}$, i.p.) was ineffective at modulating motor behaviour in the $\alpha_{2A}$AR D79N mutants. In the $\alpha_{2B}$AR and $\alpha_{2C}$AR knock-out mice, Dex produced a dose-dependent reduction in spontaneous locomotor activity with $ED_{50}$ values of $29.1 \pm 6.4$ ($P > 0.05$ vs WT control) and $57.5 \pm 11.3$ ($P > 0.05$ vs WT control) $\mu g\ kg^{-1}$, respectively.

**5** Dex was also found to produce a dose-dependent reduction in body temperature in the $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$AR WT control mice with $ED_{50}$ values of $60.6 \pm 11.0$, $16.2 \pm 2.5$ and $47.2 \pm 9.1$ $\mu g\ kg^{-1}$, i.p., respectively. In the $\alpha_{2A}$AR D79N mutants, Dex had no effect on body temperature at a dose (100 $\mu g\ kg^{-1}$, i.p.) that produced a significant reduction ($-6.2 \pm 0.5$°C; $P < 0.01$ vs vehicle) in temperature in WT controls. However, higher doses of Dex (300 and 1000 $\mu g\ kg^{-1}$, i.p) produced a small, but statistically significant decrease in temperature corresponding to $-1.7 \pm 0.4$°C and $-2.4 \pm 0.3$°C (both $P < 0.01$ vs vehicle), respectively. In the $\alpha_{2B}$AR and $\alpha_{2C}$AR knock-out mice, Dex produced a dose-dependent reduction in body temperature with $ED_{50}$ values of $28.4 \pm 4.8$ ($P > 0.05$ vs WT control) and $54.1 \pm 8.0$ ($P > 0.05$ vs WT control) $\mu g\ kg^{-1}$, respectively.

**6** In conclusion, the data are consistent with the $\alpha_{2A}$AR being the predominant subtype involved in the mediation of the antinociceptive, sedative and hypothermic actions of Dex. This profile would appear to indicate that an $\alpha_{2A}$AR subtype selective analgesic will have a narrow therapeutic window, particularly following systemic administration.

**Keywords:** $\alpha_2$-Agonist; pain; analgesia; sedation; hypothermia; tail-dip

## Introduction

$\alpha_2$-Adrenoceptors ($\alpha_2$ARs) mediate the pre- and postsynaptic inhibitory actions of the catecholamine neurotransmitter noradrenaline in the central (CNS) and peripheral nervous systems. Pharmacologically, the main functional consequences of central $\alpha_2$AR activation include the modulation of nociceptive information, motor behaviour and body temperature. In the complex control of cardiovascular function, both central and peripheral $\alpha_2$ARs are involved producing systemic hypotension and/or hypertension, respectively (see reviews by Maze & Tranquilli, 1991; Aantaa & Scheinin, 1993; Eisenach, 1994; MacDonald *et al*., 1997).

In rodents, clonidine and dexmedetomidine (Dex), which are potent and selective $\alpha_2$AR agonists, produce profound antinociception in numerous acute (Reddy *et al*., 1980; Howe *et al*., 1983; Solomon *et al*., 1989; Takano & Yaksh, 1992) and chronic (Puke & Wiesenfeld-Hallin, 1993; Lee & Yaksh, 1995; Yaksh *et al*., 1995) experimental pain models in rodents. Clinically, clonidine has been used extensively for the treatment of severe, acute (Tamsen & Gordh, 1984; Eisenach *et al*., 1989a; Mendez *et al*., 1990) and chronic (Coombs *et al*., 1986; Max *et al*., 1988; Eisenach *et al*., 1989b) pain conditions following either epidural or spinal administration. Clonidine (Bloor & Flacke, 1982) and Dex (Segal *et al*., 1988) have also been found to be useful adjuncts to general anaesthetics by reducing the anaesthetic requirement during surgery. However, while effective analgesia is obtained by these restricted routes of administration, widespread use for the treatment of pain following systemic administration is precluded by a series of adverse side-effects including sedation, profound systemic hypotension and bradycardia (Bonnet *et al*., 1989; Eisenach *et al*., 1989a; Maze & Tranquilli, 1991).

The $\alpha_2$AR has been separated into three, structurally and pharmacologically distinct subtypes: $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$AR (for reviews see Ruffolo *et al*., 1991; Bylund *et al*., 1992). These

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correspond to the cloned $\alpha_2$-C10, $\alpha_2$-C2 and $\alpha_2$-C4 adrenoceptors, designated according to human chromosomal localization (Kobilka *et al*., 1987; Regan *et al*., 1988; Weinshank *et al*., 1990) with orthologues (RG20, RNG and RG10) in the rat (Chalberg *et al*., 1990; Zeng *et al*., 1990; Lanier *et al*., 1991). The $\alpha_{2D}$AR subtype was determined to be a rat homologue of the human $\alpha_{2A}$AR. All three main subtypes are widely distributed in the nervous system although $\alpha_{2A}$AR and $\alpha_{2C}$AR subtypes appear to predominate in the CNS (Handy *et al*., 1993; Nicholas *et al*., 1993; Berkowitz *et al*., 1994). The $\alpha_{2B}$AR subtype is present in many peripheral tissues (Handy *et al*., 1993; Berkowitz *et al*., 1994) as well as in low abundance in some discrete areas of the CNS (Nicholas *et al*., 1993). $\alpha_2$AR mRNA and/or protein for individual subtypes has been found in peripheral and central sites associated with the transfer of sensory, nociceptive information, including the dorsal root ganglion (DRG; $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$AR), superficial laminae of the spinal cord ($\alpha_{2A}$), periaqueductal grey and various medullary and thalamic nuclei ($\alpha_{2A}$ and $\alpha_{2C}$AR) (Nicholas *et al*., 1993; Rosin *et al*., 1996; Talley *et al*., 1996; Gold *et al*., 1997). On the basis of this anatomical information each subtype has been implicated in the antinociceptive effects of $\alpha_2$AR-agonists.

In animal models of either acute nociception or chronic nerve/tissue injury, there is evidence to support the involvement of the $\alpha_{2A}$AR (Millan, 1992; Millan *et al*., 1994) and $\alpha_{2C}$AR (Khasar *et al*., 1995) subtype in the antinociception produced by $\alpha_2$AR-agonists. In contrast, the $\alpha_{2B}$AR (Khasar *et al*., 1995) has been primarily implicated in the peripheral hyperalgesic properties of noradrenaline released in response to tissue/nerve injury (Levine *et al*., 1986; Taiwo *et al*., 1990). A recent study showing mRNA expression for the $\alpha_{2B}$AR in the dorsal root ganglion (DRG) has suggested that this subtype may also play a role in the spinal antinociceptive effects of $\alpha_2$AR-agonists (Gold *et al*., 1997). However, a factor that complicates interpretation of these findings is that all previous pharmacological studies have used either agonists or antagonists with selectivity for the $\alpha_2$AR but with either marginal or no subtype selectivity. Moreover, it has also been suggested that, since many of these drugs have a common imidazoline moiety, a possible additional interaction at the putative imidazoline $I_1$ or $I_2$ site cannot be excluded (for review see Codd *et al*., 1995).

The aim of the present study, therefore, was to assess the relative contribution of the $\alpha_2$AR subtype(s) to the antinociception, sedation and hypothermia produced by Dex. The recent availability of mouse strains with either a point mutation, D79N, in the $\alpha_{2A}$AR gene (MacMillan *et al*., 1996) or null mutation ($\alpha_{2B}/\alpha_{2C}$AR) (Link *et al*., 1996), allows us to delineate the involvement of specific $\alpha_2$AR subtypes in the absence of selective ligands. These animals have been previously used in assessing the relative contribution of the three subtypes towards the cardiovascular properties observed with Dex and clonidine (MacMillan *et al*., 1996; Link *et al*., 1996). In these studies, the $\alpha_{2A}$AR was found to be the predominant subtype involved in the central hypotensive action of these agents (MacMillan *et al*., 1996). In contrast, the $\alpha_{2B}$AR mediated a transient, pressor effect (Link *et al*., 1996). Arguably, if either the $\alpha_{2B}$ or $\alpha_{2C}$AR subtypes contribute to the antinociceptive action of $\alpha_2$-agonists and are devoid of sedative and hypothermic properties, an $\alpha_2$AR analgesic could be produced with an acceptable therapeutic window following systemic administration. A preliminary account of this work has been presented to the British Pharmacological Society (Hunter *et al*., 1997).

## Methods

The following experimental procedures for the surgical preparation and testing of animals were reviewed and approved by the Institute Animal Care and Use Committee at Roche Bioscience.

### *Animals*

The role of the $\alpha_{2A}$AR subtype was investigated in a mouse (female, 20–30 g at the time of testing) strain (129SvJ) expressing the point mutation, D79N, in the receptor (MacMillan *et al*., 1996); 129SvJ wild type (WT) control mice were purchased from Jackson Laboratories. Mice containing a null mutation for either the $\alpha_{2B}$AR (129SvJ/C57BL) or $\alpha_{2C}$AR (129SvJ/FVB) gene (Link *et al*., 1996) were on mixed genetic backgrounds. Thus, $\alpha_{2B}$AR or $\alpha_{2C}$AR knock-out heterozygotes were bred and the offspring genotyped by means of standard Southern blotting and PCR techniques. Breeding colonies were established with either homozygous knock-out (−/−) or WT (+/+) control animals.

### *Tail immersion test*

For the studies of acute, thermal nociception, the tail-immersion test was used in which the last 3.5 cm of the tail was dipped into a water bath at 52°C and the latency to flutter the tail taken as the end-point. The maximal latency allowed was 15 s to avoid potential tissue damage.

### *Spontaneous locomotor activity*

Motor activity boxes consisted of fourteen standard perspex transfer cages. Each cage was surrounded by an Automated Cage Activity System (San Diego Instruments) containing a one inch band of photobeams and photosensors numbering 3 per box. The number of photobeam breaks were analysed by PC computer by use of PAS software. In order to assess drug action against optimal locomotor and exploratory behaviour, at a time convenient to the operator, the mice were housed under reversed 12 h light : dark cycle conditions and tested in an enclosed room illuminated with only dim red light. Vehicle or drug was administered to groups of 8–10 mice and motor activity assessed, 60 min post-drug administration, by placing the mice in the computerized cages for a period of 15 min. The number of activity counts, i.e., two or more beams broken in succession, was then recorded for each mouse and the mean ± s.e. mean calculated for each group. The treatment groups were balanced across the fourteen activity boxes and across all of the test sessions.

### *Body temperature*

Body temperature (°C) was recorded with a Yellow Springs rectal thermometer probe. Groups of 10 mice were administered either vehicle (distilled water) or dexmedetomidine as a 60 min pretreatment before the test.

### *Drugs*

Dexmedetomidine was synthesized in the Department of Medicinal Chemistry at Roche Bioscience (Palo Alto, CA, U.S.A.). Morphine was obtained from Sigma. In all experiments, drugs were administered intraperitoneally in a dose volume of 10 ml $kg^{-1}$.

### *Statistics*

All data were analysed by use of a Kruskal-Wallis one-way ANOVA followed by a pairwise comparison between vehicle and each test group with Fisher's LSD and Dunn's Procedure on the ranked data. Drug effects were considered to be statistically significant only if they were different from both the pre-dose data and the vehicle data (at that time point) at the $P<0.05$ level.

The % maximum possible effect was calculated for each animal in the form of $100\times((\text{postdose-predose})/(15\text{-predose}))$. Potency ($ED_{50}$) values were estimated by use of the following sigmoidal model: % maximum possible effect = min + (max-min)/(1 + exp(($ED_{50}$-dose)/N)), where $ED_{50}$ is the concentra-

tion for the compound to achieve half of the maximum response in the dose response curve, N is the curvature parameter and max is the maximum response. Nonlinear curve fitting was done with SAS (SAS Institute Inc., NC, U.S.A.).

## Results

### *Tail immersion test (52°C water bath)*

Dex (10–300 $\mu$g kg$^{-1}$, i.p.), 60 min post-dose, produced dose-dependent antinociception in 129SvJ WT control mice with an $ED_{50}$ value (mean ± s.e. mean, $n=10$) of 99.9 ± 14.5 $\mu$g kg$^{-1}$ (i.p.). The minimum effective dose (first dose to produce a statistically significant effect) was 100 $\mu$g kg$^{-1}$ (Dex: 10.8 ± 1.5 s vs vehicle: 3.8 ± 0.2 s; Figure 1a). The next dose, 300 $\mu$g kg$^{-1}$, produced a maximum antinociceptive effect with all animals reaching the 15.0 s cut-off. In contrast, in the $\alpha_{2A}$AR mutant (D79N) mice, Dex (100–1000 $\mu$g kg$^{-1}$, i.p., $n=8-10$) was completely ineffective as an antinociceptive agent at the maximal dose (1000 $\mu$g kg$^{-1}$, i.p.) tested (Dex: 5.0 ± 0.5 s vs vehicle: 4.4 ± 0.5 s; Figure 1a).

In comparison with the $\alpha_{2A}$AR mutants, Dex (10–300 $\mu$g kg$^{-1}$, i.p., $n=8-10$) caused a dose-dependent antinociceptive effect in both the $\alpha_{2B}$AR (Figure 1b) and $\alpha_{2C}$AR (Figure 1c) WT control and knock-out mice. The $ED_{50}$ values for the $\alpha_{2B}$AR WT and knock-out animals were 94.6 ± 17.8 and 85.9 ± 15.0 $\mu$g kg$^{-1}$ (i.p.; $n=10$ per group), respectively. For the $\alpha_{2C}$ AR WT and knock-out animals the respective $ED_{50}$ values were 116.0 ± 17.1 and 226.0 ± 62.7 $\mu$g kg$^{-1}$ (i.p.; $n=10$ per group).

Morphine (0.1–10 mg kg$^{-1}$, i.p.), used as a positive control (Luttinger, 1985), produced an antinociceptive effect in both WT (129SvJ) and $\alpha_{2A}$AR D79N mutant mice with $ED_{50}$ values of 1.8 ± 0.3 and 5.7 ± 0.6 mg kg$^{-1}$, respectively. Morphine also produced a dose-dependent antinociceptive effect in the $\alpha_{2B}$AR (WT: 6.0 ± 0.1 vs KO: 5.9 ± 0.2 mg kg$^{-1}$, i.p., $P>0.05$) and $\alpha_{2C}$AR (WT: 3.3 ± 0.3 vs KO: 4.2 ± 0.7 mg kg$^{-1}$, i.p., $P>0.05$) WT animals and their respective knock-outs.

### *Spontaneous locomotor activity*

Dex (10–1000 $\mu$g kg$^{-1}$, i.p., $n=8$) was then examined for effects on locomotor activity (Figure 2). Dex (10–300 $\mu$g kg$^{-1}$) produced a dose-dependent reduction in locomotor activity in the 129SvJ WT control mice ($ED_{50}$ 60.6 ± 10.0 $\mu$g kg$^{-1}$, i.p., $n=8$) but was ineffective in the corresponding $\alpha_{2A}$AR D79N mutants (Dex: 43.4 ± 6.5 counts even at a dose of 1000 $\mu$g kg$^{-1}$ vs vehicle: 57.4 ± 6.9 counts, $P>0.05$; Figure 2a). Dex produced a dose-dependent reduction in locomotor activity in the $\alpha_{2B}$AR ($ED_{50}$ WT: 23.5 ± 7.1 vs KO: 29.1 ± 6.4 $\mu$g kg$^{-1}$, $P>0.05$) and $\alpha_{2C}$AR ($ED_{50}$ WT: 32.3 ± 4.6 vs KO: 57.5 ± 11.3 $\mu$g kg$^{-1}$, $P>0.05$) WT controls and their respective knock-outs (Figure 2b and c).

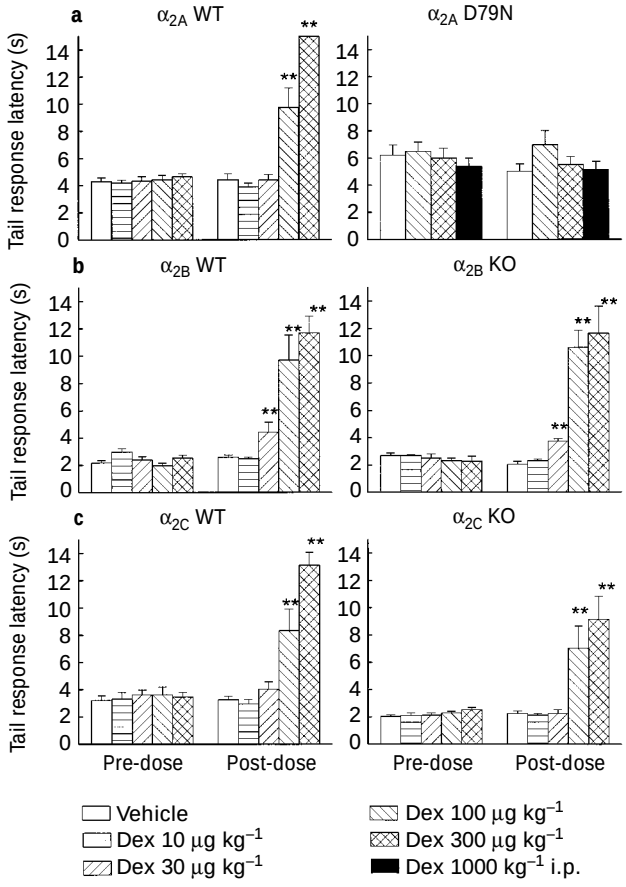


**Figure 1** Dexmedetomidine (Dex, 10–1000 $\mu$g kg$^{-1}$, i.p.) mediated, dose-dependent antinociception in the mouse tail immersion test carried out at 52°C. Dex effects in wild type (WT) control animals are shown in the left hand panel with corresponding effects in either the $\alpha_{2A}$AR D79N mutants (a), $\alpha_{2B}$AR (b) or $\alpha_{2C}$AR (c) knock-out animals shown in the right hand panel. Dex was administered 60 min before the test and compared to both the vehicle group at that time point and the respective pre-dose data. Each column represents the mean ± s.e. mean for groups of 8–10 animals. Drug effects were considered to be statistically significant only when they differed from both pre-dose data and the vehicle group at that time point. $^{**}P<0.01$.

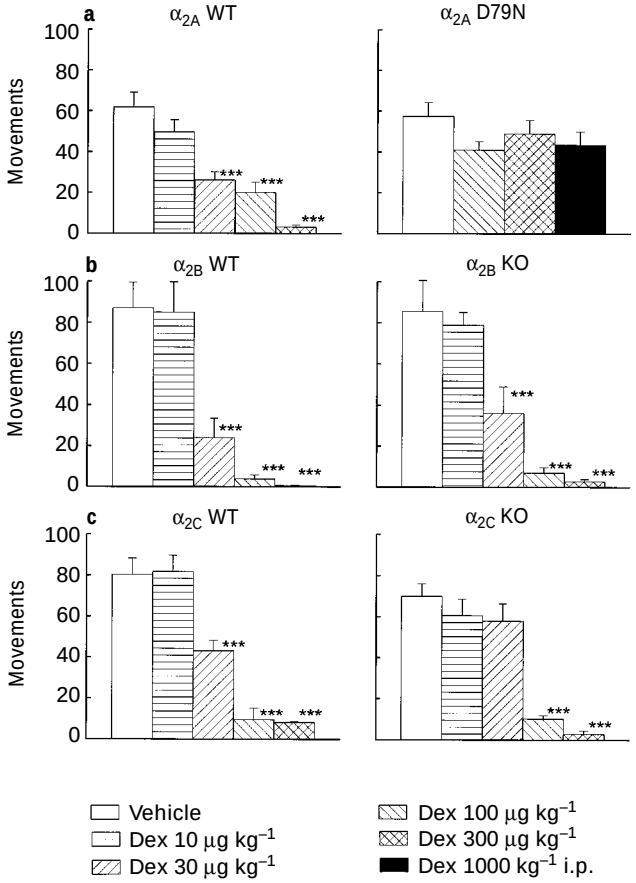


**Figure 2** Dexmedetomidine (Dex; 10–1000 $\mu$g kg$^{-1}$, i.p.) mediated, dose-dependent reduction in the computerized locomotor activity test (total number of photobeam breaks per 15 min test period). Dex effects in wild type (WT) control animals are shown in the left hand panel with corresponding effects in either the $\alpha_{2A}$AR D79N mutants (a), $\alpha_{2B}$AR (b) or $\alpha_{2C}$AR (c) knock-out animals shown in the right hand panel. Dex was administered 60 min before the test and compared to the vehicle group. Each column represents the mean ± s.e. mean for groups of 8–10 animals. $^{***}P<0.001$.

*Body temperature*

Dex (10–300 $\mu$g kg$^{-1}$, i.p.) produced a dose-dependent reduction in body temperature in the 129SvJ WT control mice ($ED_{50}$ 60.6±11.0 $\mu$g kg$^{-1}$, $n=8$) with a maximal change of −8.4±0.5°C between pre- and post-dose determinations for the group involving the highest dose, 300 $\mu$g kg$^{-1}$ (Figure 3a). In addition, the effect of Dex at this dose, 60 min following administration, was also significantly different from the vehicle group (Dex: 30.4±0.4°C vs vehicle: 38.5±0.2°C, $P<0.01$). Conversely, in the corresponding $\alpha_{2A}$AR D79N mutants, Dex was ineffective at a dose of 100 $\mu$g kg$^{-1}$ (i.p.). However, higher doses of Dex (300 and 1000 $\mu$g kg$^{-1}$, i.p., $n=8$) produced a small, but statistically significant ($P<0.01$ vs vehicle group), reduction in temperature (Figure 3a) corresponding to a maximal change of −1.7±0.4°C and −2.4±0.3°C, respectively, from the pre-dose value in each group. Dex also produced a dose-dependent reduction in body temperature in the $\alpha_{2B}$AR ($ED_{50}$ WT: 16.2±2.5 vs KO: 28.4±4.8 $\mu$g kg$^{-1}$, i.p., $P>0.05$) and $\alpha_{2C}$AR (WT: 47.2±9.1 vs KO: 54.1±8.0 $\mu$g kg$^{-1}$, i.p., $P>0.05$) WT controls and their corresponding knock-outs (Figure 3b and c). The maximal change in temperature between pre- and post-dose values in the $\alpha_{2B}$AR knock-out group was −7.8±0.3°C (Figure 3b) and in the $\alpha_{2C}$AR knock-outs was −8.4±0.5°C (Figure 3c), for the highest dose of Dex tested (300 $\mu$g kg$^{-1}$). A similar maximal change in temperature was observed at this dose in the corresponding WT controls.

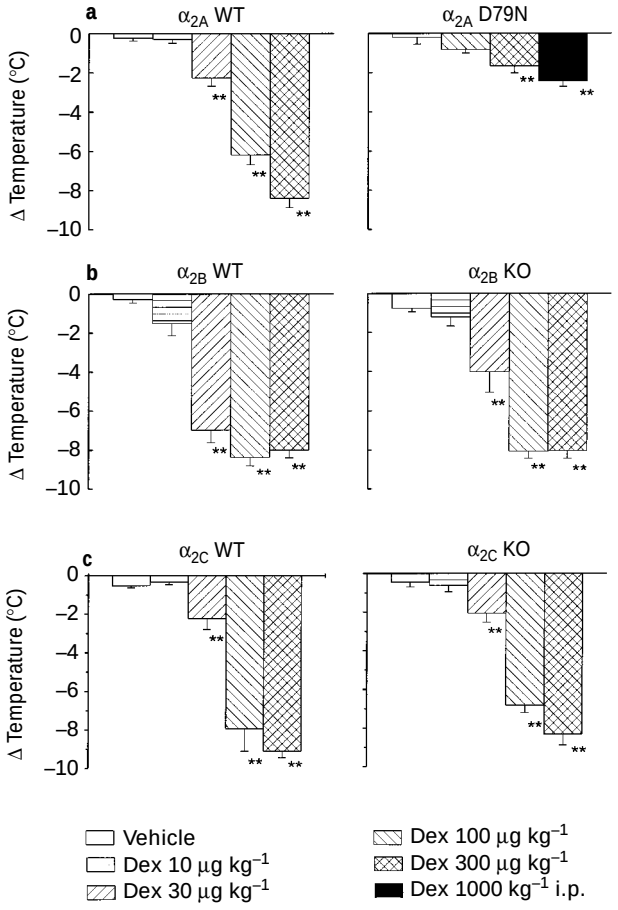


**Figure 3** Dexmedetomidine (Dex; 10–1000 $\mu$g kg$^{-1}$, i.p.) mediated, dose-dependent reduction in body temperature. Dex effects in wild type (WT) control animals are shown in the left hand panel with corresponding effects in either the $\alpha_{2A}$AR D79N mutants (a), $\alpha_{2B}$AR (b) or $\alpha_{2C}$AR (c) knock-out animals shown in the right hand panel. Dex was administered 60 min before the test and compared to both the vehicle group at that time point and the respective pre-dose data. Each column represents the mean±s.e. mean for the change (△) in temperature between pre-dose and post-dose in each individual group ($n=8$). Drug effects were considered to be statistically significant only when they differed from both pre-dose data and the post-dose vehicle group. **$P<0.01$.

## Discussion

By use of a combination of pharmacology and molecular genetics, the present study has demonstrated that the $\alpha_{2A}$AR appears to be the predominant $\alpha_2$AR subtype involved in the mediation of acute antinociception in mice. The data obtained, therefore, should resolve some of the existing confusion and controversy surrounding the issue of which $\alpha_2$AR subtypes(s) contribute towards the antinociceptive properties of $\alpha_2$-agonists, at least against an acute, noxious thermal stimulus. The present observations confirm and extend those of previous pharmacological studies in mice (Millan, 1992; Millan *et al*., 1994) which, although suggesting the importance of the $\alpha_{2A}$AR, used primarily non-selective pharmacological ligands and, therefore, ultimately could not exclude the possible involvement of the other $\alpha_2$AR subtypes, $\alpha_{2B}$ and $\alpha_{2C}$.

It has recently been emphasized that targeting the disruption of a particular gene to examine the functional role of a specific protein of interest may also trigger a series of compensatory changes in the background genotype, that may be reflected in either masking of the functional outcome of the mutation or secondary changes in the phenotype (Gerlai, 1996). This might include, for example, upregulation of a linked background gene that encodes a receptor, perhaps even a receptor subtype from the same family, that couples to a common signal transduction pathway. Consequently, the presence or absence of phenotypic abnormalities might be wrongly attributed to the null mutation when, instead, they may simply be the product of polymorphism in the genetic background (Gerlai, 1996). However, until exhaustive trials are conducted on either the null or D79N mutants to examine this issue in much more detail, the simplest explanation for the lack of effect of either the $\alpha_{2B}$AR or $\alpha_{2C}$AR null mutation on the antinociceptive response to Dex, still remains the presence of the $\alpha_{2A}$AR.

At least two caveats remain; one is that applying molecular genetics to this problem necessitated the use of mice and, therefore, it is possible that a different outcome might be obtained in another species such as rat. For example, most evidence used in support of the $\alpha_{2B}$AR and $\alpha_{2C}$AR subtypes in $\alpha_2$-mediated antinociception has been obtained in rats (Khasar *et al*., 1995; Gold *et al*., 1997). A further caveat is that such evidence has also primarily concentrated on an assessment of hyperalgesia and/or nociceptor sensitization following a chronic, inflammatory stimulus or nerve injury (*c.f.* acute nociception). *In situ* hybridization and/or immunohistochemical studies have demonstrated the presence of either mRNA or protein, respectively, for the $\alpha_{2B}$AR and $\alpha_{2C}$AR and, to a more controversial extent, $\alpha_{2A}$AR, in the rat DRG (Nicholas *et al*., 1993; Gold *et al*., 1997). Some functional studies support the molecular biology and implicate both $\alpha_{2B}$AR and $\alpha_{2C}$AR involvement in noradrenaline (NA)-mediated regulation of nociceptive activity within the primary afferent neurone (Khasar *et al*., 1995). Thus, $\alpha_{2C}$ARs located on the peripheral terminals of the primary afferent neurones have been proposed to mediate the peripheral antinociceptive effects of NA following tissue injury. In contrast, the situation for the $\alpha_{2B}$AR appears to be more complex; $\alpha_{2B}$ARs located on the terminals of sympathetic postganglionic neurones (Khasar *et al*., 1995; Gold *et al*., 1997) have been suggested to mediate NA-induced peripheral hyperalgesia, associated with tissue/nerve injury (Levine *et al*., 1986; Taiwo *et al*., 1990; Sato & Perl, 1991), through the release of prostanoids that subsequently activate/sensitize the peripheral terminals of the primary afferent neurone. Recent molecular biological evidence demonstrating the presence of the $\alpha_{2B}$AR in the DRG has proposed that these receptors, which will ultimately be located on the central

terminals on the primary afferent neurone, may mediate a direct, spinal antinociceptive effect of NA. However, once again, interpretation of the data from the functional studies is complicated by the use of non-selective agonists and antagonists to differentiate between individual $\alpha_2$AR subtypes.

While the present study does not address the issue of $\alpha_2$AR involvement in the modulation of chronic pain, we have conducted a preliminary study in rats with a peripheral nerve injury in which chronic, intrathecal administration of an antisense oligodeoxynucleotide (ODN) probe specific for the $\alpha_{2A}$AR significantly reduced the anti-allodynic activity of clonidine (Hunter *et al*., 1997). In contrast, similar application of an antisense ODN probe specific for the $\alpha_{2C}$AR was ineffective. Appropriate mismatch control ODN probes were also ineffective at modulating the anti-allodynic properties of clonidine (Hunter *et al*., 1997). However, further work is necessary to address the potential involvement of the $\alpha_{2B}$AR in this model of neuronal sensitization.

Identification of the subtype(s) responsible for the primary functional manifestations of $\alpha_2$AR activation has significant clinical implications for the viability of an $\alpha_2$AR-agonist analgesic with an acceptable therapeutic window over potential adverse events such as sedation, hypothermia and hypotension. The finding that the $\alpha_{2A}$AR also appears to be the predominant, if not exclusive, $\alpha_2$AR mediating the sedative/ataxic and hypothermic effects of Dex represents further evidence of the therapeutic limitations of an $\alpha_2$ agonist. The reduction in motor activity confirmed a previous observation with non-selective pharmacological tools (Millan *et al*., 1994). In the $\alpha_{2C}$AR knock-out animals, the absence of any observable impact on Dex-mediated reduction in locomotor activity confirmed a recent study in animals from a comparable genetic background (Sallinen *et al*., 1997). By contrast, in the same study, a small but significant attenuation in the hypothermic action of Dex was observed, which is clearly inconsistent with our own observations. At present we have no obvious explanation for this discrepancy. However, while the animals used by both groups came from the same parental line, it is possible that minor differences in the respective breeding programmes could result in effects on linked background genes leading to an altered phenotype (Gerlai, 1996).

The current use of $\alpha_2$AR agonists such as clonidine and Dex as analgesics and/or anaesthetic sparing agents has been restricted to either epidural or spinal administration. Systemic administration of these agents leads to concomitant and severe adverse events (e.g. profound sedation, hypotension and bradycardia). Collectively, the results of this study and those previously demonstrating that the $\alpha_{2A}$AR mediates the hypotensive action of Dex (MacMillan *et al*., 1996; Link *et al*., 1996) suggest that epidural or spinal administration are likely to remain the major route of administration for these drugs.

Since a number of $\alpha_2$AR agonists, contain either an imidazole or imidazoline ring moiety, it has been suggested that the antinociception produced by these agents could involve an action at either the putative $I_1$ or $I_2$ subtypes of the imidazoline site (Codd *et al*., 1995). However, the much reduced and even complete insensitivity of Dex in the $\alpha_{2A}$AR mutant mice would appear to be inconsistent with an action at either type of imidazoline receptor.

In conclusion, the present study has confirmed that, in mice, the predominant $\alpha_2$AR involved in Dex-mediated antinociception in acute models of nociception, reduction of motor behaviour and hypothermia in the $\alpha_{2A}$AR subtype. These observations illustrate the difficulty of obtaining an $\alpha_2$AR subtype selective analgesic with an acceptable therapeutic profile following systemic administration.

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