Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 457 (2002) 45–49
www.elsevier.com/locate/ejphar

Short communication

Vascular actions of 3,4-methylenedioxymethamphetamine in $\alpha_{2A/D}$-adrenoceptor knockout mice

Catherine Vandeputte, James R. Docherty*

Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland

Received 23 October 2002; accepted 29 October 2002

Abstract

We have investigated the effects of 3,4-methylenedioxymethamphetamine (MDMA) on mean arterial pressure in anaesthetised wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice. In wild-type mice, MDMA (5 mg kg^{-1}) produced a pressor response that declined to baseline by 2 min and fell below baseline to a depressor response by 5 min, whereas MDMA (20 mg kg^{-1}) produced only a pressor response that declined to baseline by 5 min. In wild-type mice, following the injection of the selective $\alpha_{2A/D}$-adrenoceptor antagonist, 2-((4,5-dihydro-1*H*-imidazole-2-yl)methyl)-2,3-di-hydro-1-methyl-1*H*-isoindole (BRL44408), the peak pressor response to MDMA (5 or 20 mg kg^{-1}) was not modified but durations of the pressor effects of both doses of MDMA were prolonged with responses significantly above baseline at 5 min. In $\alpha_{2A/D}$-adrenoceptor knockout mice, the peak response to MDMA (5 mg kg^{-1}) was similar to that in wild-type but the response fell to baseline over 5 min with no depressor component, whereas MDMA (20 mg kg^{-1}) produced a sustained pressor response significantly above baseline at 10 min. The responses were similar to those obtained in wild-type in the presence of BRL44408. It is concluded that MDMA produces depressor responses in wild-type mice by action at $\alpha_{2A/D}$-adrenoceptors to shorten the duration of the pressor response.

Keywords: (Mouse, anaesthetised); α_2-Adrenoceptor; $\alpha_{2A/D}$-Adrenoceptor knockout; MDMA (methylenedioxymethamphetamine)

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is now widely abused as a recreational drug resulting in fatalities, including some of cardiovascular origins (Dowling et al., 1987). We have previously shown that MDMA has major actions as an agonist at α_2-adrenoceptors (Lavelle et al., 1999). An α_2-adrenoceptor agonist would be predicted to have central depressor and peripheral pressor actions by analogy to agonists such as clonidine (Haeusler, 1974; Anden et al., 1976; Hamilton et al., 1980). Although it has been suggested that actions at imidazoline sites contribute to the central effects of clonidine (Tibirica et al., 1991), studies in D79N $\alpha_{2A/D}$-adrenoceptor transgenic mice report that depressor responses to clonidine or the imidazoline rilmenidine require the expression of an $\alpha_{2A/D}$-adrenoceptor (Zhu et al., 1999). In studies of anaesthetised rats, we found that the initial pressor response to MDMA (5 mg kg^{-1}) involved α_2- and possibly α_1-adrenoceptors and 5-HT_2 receptors, the pressor component at 1 min was largely α_1-adrenoceptor mediated, and the sustained depressor response involved α_2-adrenoceptors (McDaid and Docherty, 2001). Since these results suggested a major role for peripheral and central α_2-adrenoceptors, probably of the $\alpha_{2A/D}$-subtype, in the actions of MDMA in the anaesthetised rat, we chose to investigate the effects of MDMA in anaesthetised wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice.

2. Methods

Male and female C57BL6 and $\alpha_{2A/D}$-adrenoceptor (8–14 weeks, 20–30 g), obtained as breeding pairs from Jackson Laboratories, were employed in this study. The studies conform to the Declaration of Helsinki and have been approved by the Department of Health and by the RCSI Research Ethics Committee.

* Corresponding author. Tel.: +353-1-402-2269; fax: +353-1-402-2447.
E-mail address: docherty@rcsi.ie (J.R. Docherty).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02661-4

2.1. Anaesthetised mouse

Animals were anaesthetised with pentobarbitone (Sagatal) (approximately 40 mg kg^{-1}, i.p.), supplemented with pentobarbitone (approximately 10 mg kg^{-1}, i.v. per hour). Temperature was maintained at approximately 37 °C, employing a heated table. The jugular vein was exposed and cannulated for drug injection. The carotid artery was then exposed, cannulated and connected to a blood pressure transducer for pressure monitoring and recording. The duration of dissection was usually at least 1 h.

Following a 15-min equilibration period, drug administration commenced. A first injection of saline (0.2 ml) was performed and its effect was recorded over 10 min. Saline vehicle had only transient effects on mean arterial pressure. In a group of wild-type animals, the $\alpha_{2A/D}$-adrenoceptor antagonist, 2-((4,5-dihydro-1*H*-imidazole-2-yl)methyl)-2,3-di-hydro-1-methyl-1*H*-isoindole (BRL44408) (1 mg kg^{-1}), was injected and its effect was recorded for 10 min. In wild-type mice following saline or BRL44408, and in $\alpha_{2A/D}$-adrenoceptor knockout mice following saline, MDMA (5 mg kg^{-1}) was then administered intravenously and the time course of its effects on mean arterial pressure were examined over 10 min. MDMA (20 mg kg^{-1}) was then injected and the time course of its effects on blood pressure were also examined over 10 min. Blood pressure effects of MDMA were measured as the initial peak pressor response (at approximately 20–30 s post injection) and at 1, 2, 3, 4, 5 and 10 min post injection. The maximum depressor response was measured at one of these time points. In some preliminary experiments, several doses of MDMA beginning with 1 mg kg^{-1} were investigated in order to determine appropriate doses. These experiments are not reported in the results. At the end of the experiment, the animal was killed by an overdose of pentobarbitone.

2.2. Statistics

Values are expressed as mean and standard error of the mean (S.E.M.). Mean arterial pressure was calculated using this formula: diastolic blood pressure + 1/3 pulse pressure. In each set of experiments, n indicates the number of animals studied. Differences between groups were compared by analysis of variance and Dunnett's test (for comparisons with vehicle) or Bonferroni test (comparison of all groups). Means were considered significantly different when P values were <0.05.

2.3. Drugs

3,4-Methylenedioxymethamphetamine (MDMA) (Research Biochemicals, Natick, MA, USA), 2-((4,5-dihydro-1*H*-imidazole-2-yl)methyl)-2,3-di-hydro-1-methyl-1*H*-isoindole (BRL44408) (Sigma, Dublin, Ireland).

MDMA and BRL44408 were dissolved in distilled water using an ultrasonic bath and diluted in normal saline (0.9% NaCl, w/v).

3. Results

3.1. Resting haemodynamic parameters in anaesthetised mice

Resting mean arterial pressure was 66 ± 3 mm Hg ($n=13$) and 62 ± 4 mm Hg ($n=6$) in wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice, respectively. Resting heart rate was 314 ± 19 min^{-1} ($n=13$) and 274 ± 11 min^{-1} ($n=6$) in wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice, respectively. There were no significant differences between wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice in these parameters. Injection of saline (0.2 ml) induced a transient depressor effect that reached a minimum at about 5 s, then mean arterial pressure returned to the baseline level by 1 min and remained stable for the 10 min of recording. Saline injection had no significant effect on heart rate (e.g. in wild-type, baseline heart rate was 321 ± 34 min^{-1}, and 319 ± 33 min^{-1} 1 min after saline injection, $n=7$).

3.2. Effects of MDMA (5 and 20 mg kg^{-1}) on heart rate in anaesthetised mice

MDMA (5 mg kg^{-1}) induced significant increases in heart rate of 52 ± 19 ($n=6$), 49 ± 11 ($n=6$) and 61 ± 10 min^{-1} ($n=8$), whereas MDMA (20 mg kg^{-1}) induced significant increases in heart rate of 83 ± 21 ($n=5$), 88 ± 17 ($n=6$) and 96 ± 21 min^{-1} ($n=6$) in wild-type, wild-type following BRL44408 (1 mg kg^{-1}) and $\alpha_{2A/D}$-adrenoceptor knockout mice, respectively (no significant differences). Following MDMA (5 mg kg^{-1}), heart rate was not significantly above baseline by 10 min, but following MDMA (20 mg kg^{-1}), heart rate remained significantly elevated at 10 min in all three groups.

3.3. Effects of MDMA (5 and 20 mg kg^{-1}) on mean arterial pressure in anaesthetised mice

In wild-type mice, MDMA (5 mg kg^{-1}) produced a biphasic effect on mean arterial pressure, consisting of an initial pressor response falling to baseline by 2 min, and to a depressor response at about 5 min, with some recovery by 10 min (see Fig. 1). The response to MDMA (5 mg kg^{-1}) at 5 min was a significant depressor response as compared to the response to saline at 5 min ($P<0.05$). In $\alpha_{2A/D}$-adrenoceptor knockout mice, MDMA (5 mg kg^{-1}) produced an initial pressor effect on mean arterial pressure, which fell back to baseline by 5–10 min (see Fig. 1). There was no significant difference between wild-type and knockout mice in the pressor peak, but 4 min after injection, the depressor response to MDMA in wild-type was significantly different from the

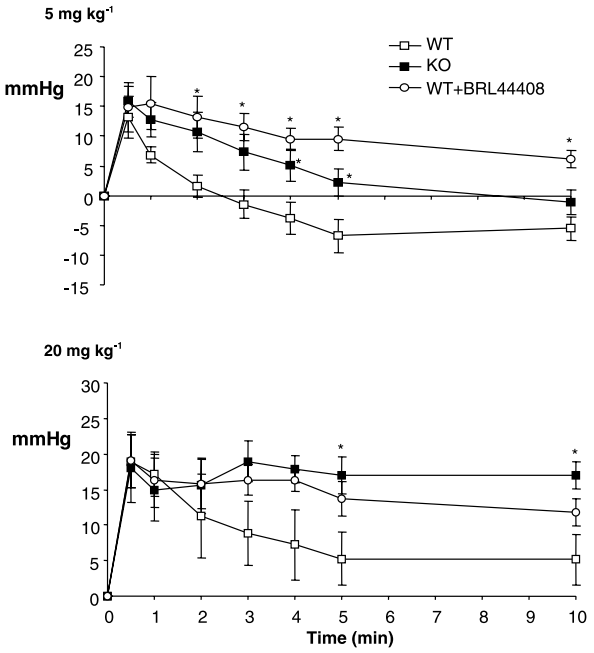

Fig. 1. Time course of the change in mean arterial pressure produced by MDMA (5 and 20 mg kg^{-1}) injected at time 0, in anaesthetised wild-type (WT), in absence or presence of BRL44408 (1 mg kg^{-1}), and $\alpha_{2A/D}$-adrenoceptor knockout mice (KO). Vertical bars represent S.E.M. from six to seven experiments. *$P<0.05$ vs. wild-type.

lack of depressor response in knockout (Fig. 1). In wild-type animals receiving BRL44408 (1 mg kg^{-1}), MDMA (5 mg kg^{-1}) induced an initial pressor response which was similar to that observed in wild-type and knockout mice. By 2 min post injection, the pressor response fell slightly but remained above the baseline, and was significantly different from the control wild-type group even at 10 min (Fig. 1).

In wild-type mice, MDMA (20 mg kg^{-1}) produced a pressor effect on mean arterial pressure, which returned to baseline at about 5 min (see Fig. 1). In $\alpha_{2A/D}$-adrenoceptor knockout mice and wild-type receiving BRL44408 (1 mg kg^{-1}), MDMA (20 mg kg^{-1}) produced a pressor effect on mean arterial pressure, which remained elevated even by 10 min (see Fig. 1). There was no significant difference between groups in the pressor peak, but by 4 min after injection, the pressor response to MDMA in knockout was significantly different from the near baseline response in wild-type. At 10 min, the effects of MDMA (20 mg kg^{-1}) were significantly greater than the effects of saline on mean arterial pressure in all groups except control wild-type (Fig. 1).

4. Discussion

The objective of this study was to confirm in anaesthetised mice using knockout technology our previous findings concerning the cardiovascular actions of MDMA in anaesthetised rat, and specifically to identify the role of $\alpha_{2A/D}$-adrenoceptors in this response.

The present results demonstrate that MDMA produces biphasic pressor/depressor responses in wild-type anaesthetised mice, but that the pressor component is prolonged and the depressor component is absent in $\alpha_{2A/D}$-adrenoceptor knockout mice or following the α_{2D}-adrenoceptor antagonist BRL44408 in wild-type mice. Specifically MDMA (5 mg kg^{-1}) had a short-lived pressor response with a later depressor component in wild-type but not in knockout, whereas MDMA (20 mg kg^{-1}) produced a short-lived pressor response in wild-type, but a sustained pressor response in knockout and BRL44408 treated wild-type mice. Hence, MDMA acts on $\alpha_{2A/D}$-adrenoceptors, presumably located centrally, to produce depressor responses (5 mg kg^{-1}) or to reverse pressor responses (20 mg kg^{-1}).

In the present study, baseline mean arterial pressure and heart rate were similar in wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice. However, other authors have found that $\alpha_{2A/D}$-adrenoceptor knockout or transgenic mice presented either an increased ($\alpha_{2A/D}$-knockout: Altman et al., 1999) or unchanged heart rate (D79N transgenic: Zhu et al., 1999). However, between these studies, there were large differences in the baseline heart rate of wild-type animals (approximately 400 versus 500 min^{-1}). There are reports of no increase in mean arterial pressure in the same strain of knockout mice as used here (Altman et al., 1999; Duka et al., 2000), whereas an increase (Zhu et al., 1999) or no change in mean arterial pressure (MacMillan et al., 1996) was detected in transgenic animals (D79N) in which the receptor is still present with a break in its transduction pathway. However, between these studies, there were large differences in the baseline mean arterial pressure of wild-type animals (range: 104–146 mm Hg). In these previous studies, haemodynamic parameters were recorded in conscious animals, the day after the catheterization. In the present study, recordings were carried out in anaesthetized animals in which the period of dissection was at least 1 h, which may explain the lowered values of heart rate and mean arterial pressure as well as the absence of differences between wild-type and knockout animals.

We have previously examined the vascular actions of MDMA in the anaesthetised rat (McDaid and Docherty, 2001). MDMA (5 mg kg^{-1}) produced a biphasic response, consisting of a pressor response followed by a depressor response. MDMA (20 mg kg^{-1}) also produced a biphasic response, but the depressor component developed much more slowly. In the anaesthetised rat, MDMA (5 mg kg^{-1}) had biphasic actions with three components: a pressor response with two components (initial peak and a secondary response at 1 min) and a sustained depressor response (McDaid and Docherty, 2001). By analogy with α_2-adrenoceptor agonists such as clonidine or xylazine, it might be expected that the initial pressor response to MDMA is due to action at peripheral postjunctional α_1- and α_2-adrenoceptors (Haeusler, 1974; Docherty and McGrath, 1980). The initial pressor response to MDMA was found to involve a combination of α_2- and possibly α_1-adrenoceptors and/or

5-HT_2 receptors, presumably located peripherally on vascular smooth muscle (McDaid and Docherty, 2001). The response to MDMA (5 mg kg^{-1}) at 1 min was reversed from a pressor response to a depressor response by drugs with α_1-adrenoceptor actions (McDaid and Docherty, 2001). This suggests that α_1-adrenoceptors dominate the pressor component at 1 min, so that the presumed α_2-adrenoceptor/5-HT_2 receptor components of the pressor response appear to be relatively short-lived, at least in the rat. The depressor response to MDMA in the anaesthetised rat was found to be at least partly α_2-adrenoceptor mediated (McDaid and Docherty, 2001).

Previous studies in knockout mice suggest that the central sympathoinhibitory response to α_2-adrenoceptor agonists is mediated by $\alpha_{2A/D}$-adrenoceptors (MacMillan et al., 1996; Altman et al., 1999), and in our studies, this would explain the loss of the depressor response to MDMA in $\alpha_{2A/D}$ knockout mice. However, although Link et al. (1996) found that the pressor response to α_2-adrenoceptor agonists was absent in α_{2B}-adrenoceptor knockout mice, MacMillan et al. (1996) found that both pressor and depressor responses to α_2-adrenoceptor agonists were markedly reduced in $\alpha_{2A/D}$ (D79N) transgenic mice. Clearly, the simultaneous pressor and depressor responses to α_2-adrenoceptor agonists involving $\alpha_{2A/D}$-adrenoceptors could explain these contradictory findings. In our study of MDMA, peak pressor responses were unaltered in $\alpha_{2A/D}$-adrenoceptor knockout mice. However, MDMA produces pressor responses in the anaesthetised rat via α_1-, α_2- and perhaps 5-HT_2 receptor stimulation, so that it is likely that the initial pressor response in the anaesthetised mouse involves actions at α_1-adrenoceptors and possibly α_2-(non-$\alpha_{2A/D}$)-adrenoceptors and 5-HT_2 receptors, which are presumably located peripherally.

In man, cardiovascular actions of MDMA have not been widely studied, but it has been reported generally to produce a rise in blood pressure (Grob et al., 1996; Vollenweider et al., 1998) and tachycardia (Hayner and McKinney, 1986). However, Downing (1986) reported an initial rise in blood pressure, but also a tendency for blood pressure to fall below baseline after several hours. Most studies were not carried out under controlled conditions in which α_2-adrenoceptor actions could be investigated. It is likely that MDMA in recreational doses has effects in man similar to the 1 mg kg^{-1} dose in the rat: dominant pressor actions which mask the α_2-adrenoceptor mediated depressor actions. Further information is needed to elucidate the effects of MDMA on blood pressure in man. MDMA is linked to cardiovascular mortality (Dowling et al., 1987), but has not been widely studied. Certainly, chronic use of methamphetamine may also result in serious cardiovascular changes in man including tachycardia and palpitations (Chan et al., 1994), and another amphetamine derivative, fenfluramine, has been linked to valvular heart disease (Connolly et al., 1997).

It is feasible that α_2-adrenoceptor agonism contributes to the central abusive and peripheral cardiovascular and autonomic side effects of MDMA. For instance, it has been reported that MDMA decreases firing rates of serotonergic and noradrenergic but not dopaminergic neurones in the rat dorsal and median raphe (Piercey et al., 1990): an action at prejunctional α_2-adrenoceptors is likely.

In conclusion, in anaesthetised mice, MDMA has significant $\alpha_{2A/D}$-adrenoceptor agonist actions that contribute to the rapid decline in the pressor response to a depressor response; these $\alpha_{2A/D}$-adrenoceptors are presumably located centrally. The initial transient pressor response may involve actions at α_1-adrenoceptors and possibly α_2-(non-$\alpha_{2A/D}$)-adrenoceptors and 5-HT_2 receptors, which are presumably located peripherally.

Acknowledgements

Supported by the Enterprise Ireland, Science and Technology Against Drugs Initiative, the Irish Heart Foundation and the Health Research Board.

References

Altman, J.D., Trendelenburg, A.U., MacMillan, L., Bernstein, D., Limbird, L., Starke, K., Kobilka, B.K., Hein, L., 1999. Abnormal regulation of the sympathetic nervous system in α_{2A}-adrenergic receptor knockout mice. Mol. Pharmacol. 56, 154–161.

Anden, N.E., Grabowska, M., Strombom, U., 1976. Different alpha-adrenoreceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. Naunyn-Schmiedeberg's Arch. Pharmacol. 292, 43–52.

Chan, P., Chen, J.H., Lee, M.H., Deng, J.F., 1994. Fatal and non-fatal methamphetamine intoxication in the intensive care unit. J. Toxicol., Clin. Toxicol. 32, 147–155.

Connolly, H.M., Crary, J.L., McGoon, M.D., Hensrud, D.D., Edwards, B.S., Edwards, W.D., Schaff, H.V., 1997. Valvular heart disease associated with fenfluramine-phentermine. N. Engl. J. Med. 337, 581–588.

Docherty, J.R., McGrath, J.C., 1980. A comparison of pre- and postjunctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat: evidence for two types of postjunctional alpha-adrenoceptor. Naunyn-Schmiedeberg's Arch. Pharmacol. 312, 107–116.

Dowling, G.P., McDonough, E.T., Bost, R.O., 1987. 'Eve' and 'ecstasy'. A report of five deaths associated with the use of MDEA and MDMA. J. Am. Med. Assoc. 257, 1615–1617.

Downing, J., 1986. The psychological and physiological effects of MDMA on normal volunteers. J. Psychoact. Drugs 18, 335–340.

Duka, I., Gavras, I., Johns, C., Handy, D.E., Gavras, H., 2000. Role of the postsynaptic α_2-adrenergic receptor subtypes in catecholamine-induced vasoconstriction. Gen. Pharmacol. 24, 101–106.

Grob, C.S., Poland, R.E., Chang, L., Ernst, T., 1996. Psychobiologic effects of 3,4-methylenedioxymethamphetamine in humans: methodological considerations and preliminary observations. Behav. Brain Res. 73, 103–107.

Haeusler, G., 1974. Clonidine-induced inhibition of sympathetic nerve activity: no indication for a central presynaptic or an indirect sympathomimetic mode of action. Naunyn-Schmiedeberg's Arch. Pharmacol. 286, 97–111.

Hamilton, T.C., Hunt, A.A.E., Poyser, R.H., 1980. Involvement of central alpha2-adrenoceptors in the mediation of clonidine-induced hypotension in the cat. J. Pharm. Pharmacol. 32, 788–789.

Hayner, G.N., McKinney, H., 1986. The dark side of ecstasy. J. Psychoact. Drugs 18, 341–346.

Lavelle, A., Honner, V., Docherty, J.R., 1999. Investigation of the prejunctional alpha2-adrenoceptor mediated actions of MDMA in rat atrium and vas deferens. Br. J. Pharmacol. 128, 975–980.

Link, R.E., Desai, K., Hein, L., Stevens, M.E., Chruscinski, A., Bernstein, D., Barsh, G.S., Kobilka, B.K., 1996. Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. Science 273, 803–805.

MacMillan, L.B., Hein, L., Smith, M.S., Piascik, M.T., Limbird, L.E., 1996. Central hypotensive effects of the α_{2A}-adrenergic receptor subtype. Science 273, 801–803.

McDaid, J., Docherty, J.R., 2001. Vascular actions of MDMA involve α_1 and α_2-adrenoceptors in the anaesthetized rat. Br. J. Pharmacol. 133, 429–437.

Piercey, M.F., Lum, J.T., Palmer, J.R., 1990. Effects of MDMA ('ecstasy') on firing rates of serotonergic, dopaminergic and noradrenergic neurones in the rat. Brain Res. 526, 203–206.

Tibirica, E., Feldman, J., Mermet, C., Gonon, F., Bousquet, P., 1991. An imidazoline-specific mechanism for the hypotensive effect of clonidine: a study with yohimbine and idazoxan. J. Pharmacol. Exp. Ther. 256, 606–613.

Vollenweider, F.X., Gamma, A., Liechti, M., Huber, T., 1998. Psychological and cardiovascular effects and short-term sequelae of MDMA ('ecstasy') in MDMA-naïve healthy volunteers. Neuropsychopharmacology 19, 241–251.

Zhu, Q.M., Lesnick, J.R., MacLennan, S.J., Dillon, M.P., Eglen, R.M., Blue Jr., D.R., 1999. Cardiovascular effects of rilmenidine, moxonidine and clonidine in conscious wild-type and D79N α_{2A}-adrenoceptor transgenic mice. Br. J. Pharmacol. 126, 1522–1530.