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Deletion of the adenosine A_{2A} receptor in mice enhances spinal cord neurochemical responses to an inflammatory nociceptive stimulus

M.J. Hussey[1], G.D. Clarke, C. Ledent[2], I. Kitchen, S.M.O. Hourani*

Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, UK

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ABSTRACT

Knockout mice lacking the adenosine A_{2A} receptor are less sensitive to nociceptive stimuli, and this may be due to the presence of pronociceptive A_{2A} receptors on sensory nerves. In support of this hypothesis, we have recently shown that in A_{2A} receptor knockout mice there are marked reductions in the changes of two markers of spinal cord neuronal activity, [^{3}H]MK801 binding to NMDA receptors and uptake of [^{14}C]-2-deoxyglucose, in response to formalin injection. We now report that following a more prolonged inflammatory stimulus, consisting of intraplantar injections of PGE_2 and paw pressure, there was in contrast an increase in [^{3}H]MK801 binding and [^{14}C]-2-deoxyglucose uptake in the spinal cords of the A_{2A} receptor knockout mice which was much greater than in the wild-type mice. This increase suggests that when there is a pronounced inflammatory component to the stimulus, loss of inhibitory A_{2A} receptors on inflammatory cells outweighs the loss of pronociceptive A_{2A} receptors on peripheral nerves so that overall there is an increase in nociceptive signalling. This implies that although A_{2A} antagonists have antinociceptive effects they may have only limited use as analgesics in chronic inflammatory pain.

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1. Introduction

The endogenous purine nucleoside adenosine has complex effects on pain pathways which depend on the receptor subtype activated. It acts through four G protein coupled receptors, called A_1, A_{2A}, A_{2B} and A_3, but at physiological levels of adenosine the A_1 and A_{2A} receptors are thought to be the most important [9–11] and they have opposing effects on pain. Whereas activation of the A_1 receptors has an antinociceptive effect, probably through inhibitory A_1 receptors in the spinal cord, activation of the A_{2A} receptor has a pronociceptive effect, which may be due to the presence of these stimulatory receptors on sensory nerves [28,29]. In support of these two opposing actions, mice in which the A_1 receptor has been genetically deleted (A_1 knockout mice) have enhanced nociceptive responses [16], whereas A_{2A} knockout mice have reduced responses to thermal nociceptive stimuli [4,12,22]. A_{2A} knockout mice also have reduced sensitivity to the nociceptive effects of intraplantar injection of formalin, with both the initial nociceptive and the delayed inflammatory phases of the response being reduced, and this was mimicked by systemic administration of the selective A_{2A} antagonist SCH58261 [14]. A similar reduction in the hyperalgesic responses to intraplantar injection of another inflammatory stimulus, carrageenan, in A_{2A} knockout mice or in wild-type mice locally injected with another A_{2A} antagonist, ZM241385, have recently been reported [23]. In this study mechanical hyperalgesia was also seen in response to intraplantar injection of an A_{2A} receptor agonist, CGS 21680, which was lost in the A_{2A} knockout [23]. These findings confirm the pronociceptive effects of A_{2A} receptors in the periphery.

As well as having altered behavioural responses to nociceptive stimuli, A_{2A} knockout mice have altered spinal cord neurochemistry. There is a large reduction in the density of the binding of [^{3}H]MK801 to NMDA glutamate receptors and a decrease in the uptake of [^{14}C]-2-deoxyglucose (a marker for neuronal activity) in the spinal cord of these mice, but no change in the binding to NK_1 receptors or AMPA receptors [14,15]. There are also changes in spinal cord opioid receptor binding in parallel with changes in the potency of opioid receptor agonists as antinociceptive agents [4]. As well as changes in the spinal cords of naïve, unchallenged mice, there is a reduced spinal cord response to nociceptive stimulus. In the wild-type mice intraplantar administration of formalin caused a reduced binding of [^{3}H]MK801 to NMDA receptors and an increased uptake of [^{14}C]-2-deoxyglucose in the spinal cord, but these changes were significantly reduced in the A_{2A} knockout mice [15]. These changes in the knockout mice are not likely to be due to loss of A_{2A} receptors in the spinal cord itself, as mRNA

* Corresponding author. Tel.: +44 01483 689707; fax: +44 01483 686401.
E-mail address: s.hourani@surrey.ac.uk (S.M.O. Hourani).
[1] Current address: Novartis Horsham Research Centre, Horsham, West Sussex RH12 5AB, UK.
[2] Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire, Université Libre de Bruxelles, B-1070, Belgium.

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for the A_{2A} receptor is not expressed in the spinal cord [18] and we have been unable to detect A_{2A} receptors there by autoradiographic techniques [4]. mRNA for the A_{2A} receptor is present in the dorsal root ganglion, although receptors have not been detected there by immunohistochemistry [17,23], suggesting that any receptors expressed may be present at the nerve terminals in the periphery. The changes seen in the spinal cords of A_{2A} knockout mice may therefore reflect reductions in nociceptive inputs to the spinal cord caused by the loss of pronociceptive peripheral A_{2A} receptors.

As well as their role in nociceptive pathways, adenosine A_{2A} receptors are also found on inflammatory cells including neutrophils, monocytes and macrophages, where adenosine plays an inhibitory role and is thought to have an anti-inflammatory effect [5]. It seemed possible therefore that knockout of the A_{2A} receptor might in some cases enhance responses to nociceptive stimuli rather than reducing them, if there was a pronounced inflammatory component. While the second phase of the response to formalin does have an inflammatory component this is a relatively short-lived response, so we looked instead at a longer inflammatory stimulus to see if we could detect such an enhancement. We used intraplantar injection of prostaglandin E_2 (PGE_2) combined with paw pressure over a period of 3 h, and looked at spinal cord responses over a 24 h period. PGE_2 is a pronociceptive inflammatory mediator, and peripheral injection of PGE_2 causes hyperalgesia that lasts for up to 4 h [see 1–3, 20]. In addition, PGE_2 can have pro-inflammatory effects, and acting via the EP4 receptor it plays a role in the pathogenesis of rheumatoid arthritis [13,24]. Intraplantar PGE_2 injection followed by mechanical paw pressure (to test for hyperalgesia) has been used as a model for inflammatory pain in rats and mice, causing hyperalgeisa, allodynia and oedema lasting for longer than 60 min [6,19,26]. It should therefore provide a robust nociceptive stimulus, mimicking a longer-lasting inflammatory pain condition. We found that in some regions of the spinal cord genetic deletion of the A_{2A} receptor enhanced the responses to this more prolonged inflammatory stimulus, in contrast to our previously reported inhibition of the responses to formalin, suggesting that the role of A_{2A} receptors in nociception depends on the stimulus.

2. Materials and methods

Animals. All animals used in this study were wild-type and A_{2A} knockout male mice on a CD1 background [22] aged 8–12 weeks bred from heterozygotes at the University of Surrey. Animals were age matched throughout with a maximum of 1 week difference between groups of mice used for comparative experiments. All experiments described followed protocols agreed by the UK Home Office in accordance with the Animals (Scientific Procedures) Act 1985, UK.

PGE_2 injection and mechanical paw pressure. Mice were designated to one of five experimental groupings which corresponded to the time point at which they were to be killed. The five groupings were naïve control (before treatment), $t = 0$ (immediately after the 3 h treatment) and 3, 6 and 24 h later. Mice designated to treatment groups were lightly restrained before receiving 100 ng of PGE_2 (Sigma, UK) in a volume of 5 μl into the dorsal surface of the left hind paw. PGE_2 was dissolved in ethanol and further diluted using phosphate-buffered saline so that the amount of ethanol injected was less than 1%. Mice received a total of three injections of PGE_2 into the same paw with a 1 h interval between injections. One hour after each PGE_2 injection and immediately prior to subsequent injection, the injected paw was placed under a pressure bar with a linearly increasing pressure force with a preset maximum of 250 g (Ugo-Basile analgesymeter modified by Kitchen [21]). At the appropriate time points the mice were killed by cervical dislocation and the spinal cords removed, frozen in isopentane at −25 °C and then stored at −80 °C until required and processed for receptor autoradiography. For measurement of [^{14}C]-2-deoxyglucose uptake the mice received PGE_2 injection and mechanical paw pressure as above, however 5 min before the relevant time point mice were restrained in a plastic cylinder, their tails were warmed under a heat lamp and 3700kBq/kg [^{14}C]-2-deoxyglucose (ARC, USA) was injected intravenously via the tail vein. 20 min later the mice were killed via cervical dislocation and spinal cords were dissected out, frozen in isopentane at −25 °C and then stored at −80 °C until required and processed for autoradiography.

Autoradiographic assessment of [^{3}H] *MK801 binding.* 20 μm thick sections were cut from all four anatomical regions of the spinal cord (cervical, thoracic, lumbar and sacral) using a crysostat (Microm 505E, Zeiss, UK) maintained at −20 °C and thaw-mounted onto gelatine-coated slides. Adjacent sections were cut for determination of total binding and non-specific binding (NSB). Slides with tissue sections were placed into storage boxes containing desiccant (Drierite, VWR, UK) for a period of 2 h at 4 °C and were then frozen at −20 °C before use. Sections were pre-incubated in 50 mM Tris buffer (Trizma, Sigma, UK) at pH 7.4 containing 1 μM glutamate, 1 μM glycine and 1 μM spermidine for 20 min at room temperature. Total binding was determined by incubating in the same buffer but with 70 nM [^{3}H]-MK801 (Perkin Elmer Life Sciences, USA) for 1 h at 4 °C, and NSB was determined by the addition of 1 mM unlabelled MK801. Sections were washed for a total of 60 s in three changes of ice-cold buffer before being briefly rinsed in distilled water and then dried in a stream of cool air. Slides were placed into autoradiography cassettes (G.E. Healthcare, UK) and apposed for 3 weeks to [^{3}H]-Hyperfilm (GE Healthcare, UK) alongside [^{3}H]-microscales of known radioactive concentrations (4048–3.74 Bq/mg, G.E. Healthcare, UK). The resultant autoradiograms were developed in Develex developer (Patterson Scientific, UK) for 5 min, washed in distilled water for 30 s and fixed in Amfix (Patterson Scientific, UK) for 4 min. Films were washed for 30 min in distilled water then air-dried prior to analysis.

Autoradiographic assessment of [^{14}C]-2-*deoxyglucose uptake.* 20 μm thick sections were cut from all four anatomical levels of the spinal cord (cervical, thoracic, lumbar and sacral) using a crysostat (Microm 505E, Zeiss, UK) and thaw-mounted onto gelatine-coated slides. Once dried the slides were apposed to Kodak MR-1 film alongside [^{14}C]-microscale standards of known radioactive concentration (31.89–1.11 kBq/g, G.E. Healthcare, UK). Autoradiographic exposure time was three weeks. The resultant autoradiograms were developed in Kodak D-19 developer for 75 s, washed in distilled water containing acetic acid for 30 s and fixed in Kodak rapid fixer for 3 min. Films were washed in distilled water for 30 min and then air-dried prior to analysis.

Analysis of autoradiographic images. Quantitative analysis of spinal cord autoradiographic images was performed using an MCID imaging system (Imaging Research, Canada) as previously described [14,15]. For each region of spinal cord examined, at least three sections were used for quantification and measurements were taken from the whole spinal cord section to improve accuracy and provide a more robust comparison between the genotypes. [^{3}H]MK801 binding was quantified by reference to the [^{3}H]-microscale standards and expressed as fmol/mg tissue using the calibration provided with the standards. [^{14}C]-2-deoxyglucose uptake was quantified by reference to the [^{14}C]-microscale standards and expressed as kBq/g using the calibration provided with the standards.

Data analysis and statistical procedures. Because of the different control values observed between naïve wildtype and adenosine A_{2A} receptor knockout mice reported previously [14,15], data were transformed to generate values as a percentage of control. Statistical analysis was carried out using two-way analysis of variance

(ANOVA) for factors region and genotype, with post hoc analysis using Fischer's LSD test where appropriate. One-way ANOVA followed by Dunnett's post hoc test was carried out on the raw data to identify any differences between control values and the data obtained from spinal cords taken from mice 0, 3, 6 and 24 h following PGE_2/paw pressure. A probability value of $P < 0.05$ was taken to be significant in all cases.

3. Results

We have previously reported a large decrease in [^{3}H]MK801 binding to NMDA receptors in all regions of the spinal cords of A_{2A} knockout mice compared to wild-type controls [14]. In mice treated with PGE_2/paw pressure there were no significant changes in [^{3}H]MK801 binding in the cervical and thoracic segments in either wild-type or A_{2A} receptor knockout mice, but there was a significant increase in binding at 3 h after treatment in the thoracic and lumbar regions which was greater in the A_{2A} knockout mice than in the wild-type (Fig. 1, $P < 0.05$, one-way ANOVA with Dunnett's post hoc test). Overall there was a significant effect of genotype in the lumbar and sacral regions ($P < 0.05$, two-way ANOVA).

We have also previously reported that the uptake of [^{14}C]-2-deoxyglucose in all regions of the spinal cords from A_{2A} receptor knockout mice was significantly reduced compared to wild-type mice [15]. In wild-type mice treated with PGE_2/paw pressure there was no change in [^{14}C]-2-deoxyglucose uptake in any region of the spinal cord, while in A_{2A} receptor knockout mice there was a significant increase in [^{14}C]-2-deoxyglucose uptake 3 h after treatment in the thoracic region and both immediately and 3 h after treatment in the lumbar and sacral regions ($P < 0.05$, one-way ANOVA with Dunnett's post hoc test), with an overall difference between genotypes in lumbar and sacral regions ($P < 0.05$, two-way ANOVA) (Fig. 2).

4. Discussion

The level of restraint required in the mice during the paw pressure procedure precluded reliable assessment of nociceptive responses, but the time-dependent changes in the spinal cord neurochemical markers that we observed following this procedure suggested that it did cause a nociceptive stimulus. The PGE_2/paw pressure procedure lasted for 3 h, so it represents a more long-lasting inflammatory stimulus compared to the formalin procedure used in our previous study [15], in which there was a single injection and the nociceptive behaviour only lasted for an hour.

Whereas in the formalin procedure the two markers of spinal cord activity, [^{3}H]MK801 binding and [^{14}C]deoxyglucose uptake, were greatly reduced in the A_{2A} knockout mice compared to the wild-type [15], when we studied the same markers of spinal cord activity in the PGE_2/paw pressure model an entirely different pattern of responses was observed. There was an increase in [^{3}H]MK801 binding in the lumbar and sacral segments in both wild-type and A_{2A} receptor knockout mice 3 h after the final paw pressure stimulus, 6 h after the initial PGE_2 injection. As this ligand binds to the open channel of the NMDA receptor [8] this may reflect increased channel opening, a phenomenon which plays a crucial part in the spinal cord sensitisation which is known to occur with prolonged nociceptive stimulation [7,25]. This increase was enhanced in the A_{2A} knockout mice, suggesting that in these mice there is an increased nociceptive stimulus, in contrast to the results seen with formalin. This apparently paradoxical enhancement probably reflects the role of inflammatory cells in the response to PGE_2/paw pressure, as the A_{2A} receptor is found on many inflammatory cells including neutrophils, monocytes and macrophages, where it plays an inhibitory role and is thought to have an

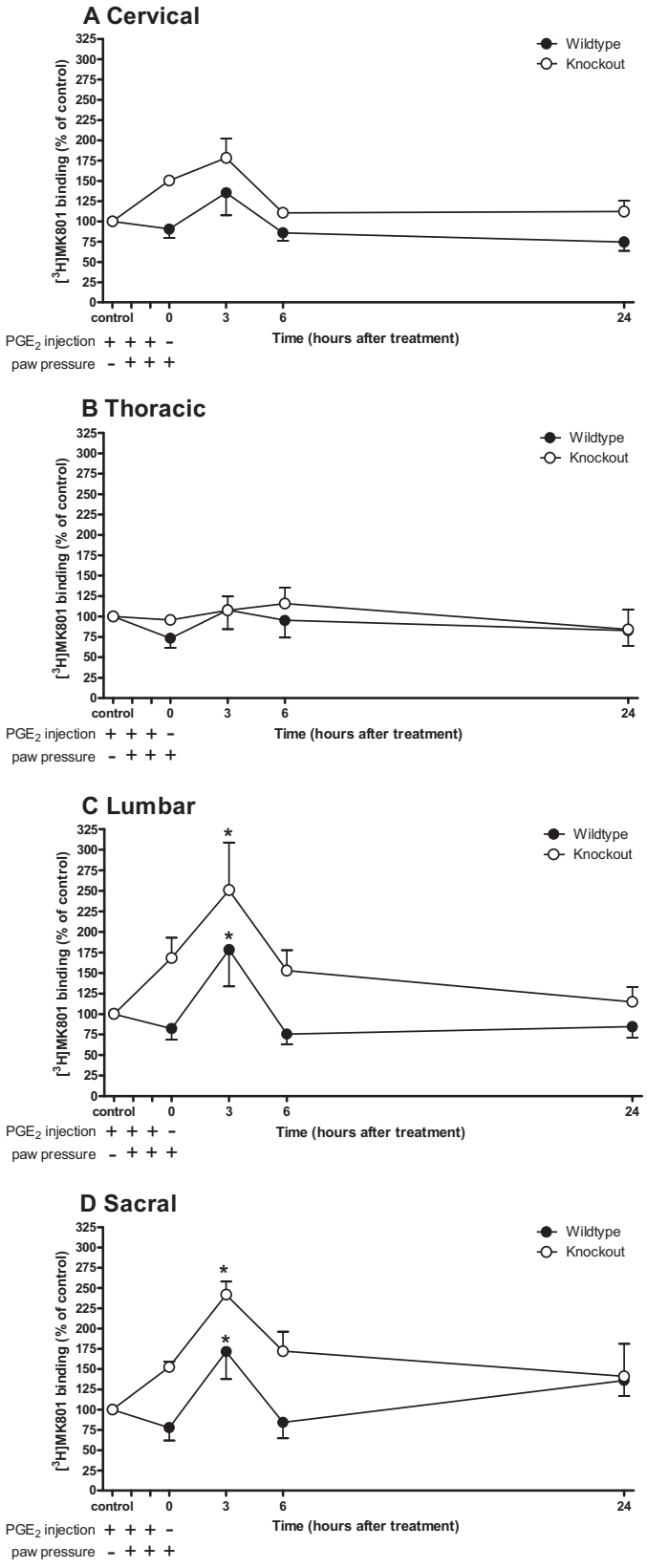

Fig. 1. [^{3}H]MK801 binding to NMDA glutamate receptors in spinal cords of wild-type and adenosine A_{2A} receptor knockout mice after repeated PGE_2 injection and mechanical paw pressure (mean ± S.E.M., $n = 3$–7). Measurements were taken from (A) cervical, (B) thoracic, (C) lumbar and (D) sacral regions, and expressed as a percentage of the values before the first injection of PGE_2. Two-way ANOVA revealed a significant genotype effect in lumbar and sacral regions ($P < 0.05$). *$P < 0.05$, time point vs control. One-way ANOVA followed by Dunnett's post hoc test on untransformed data to reveal time differences.

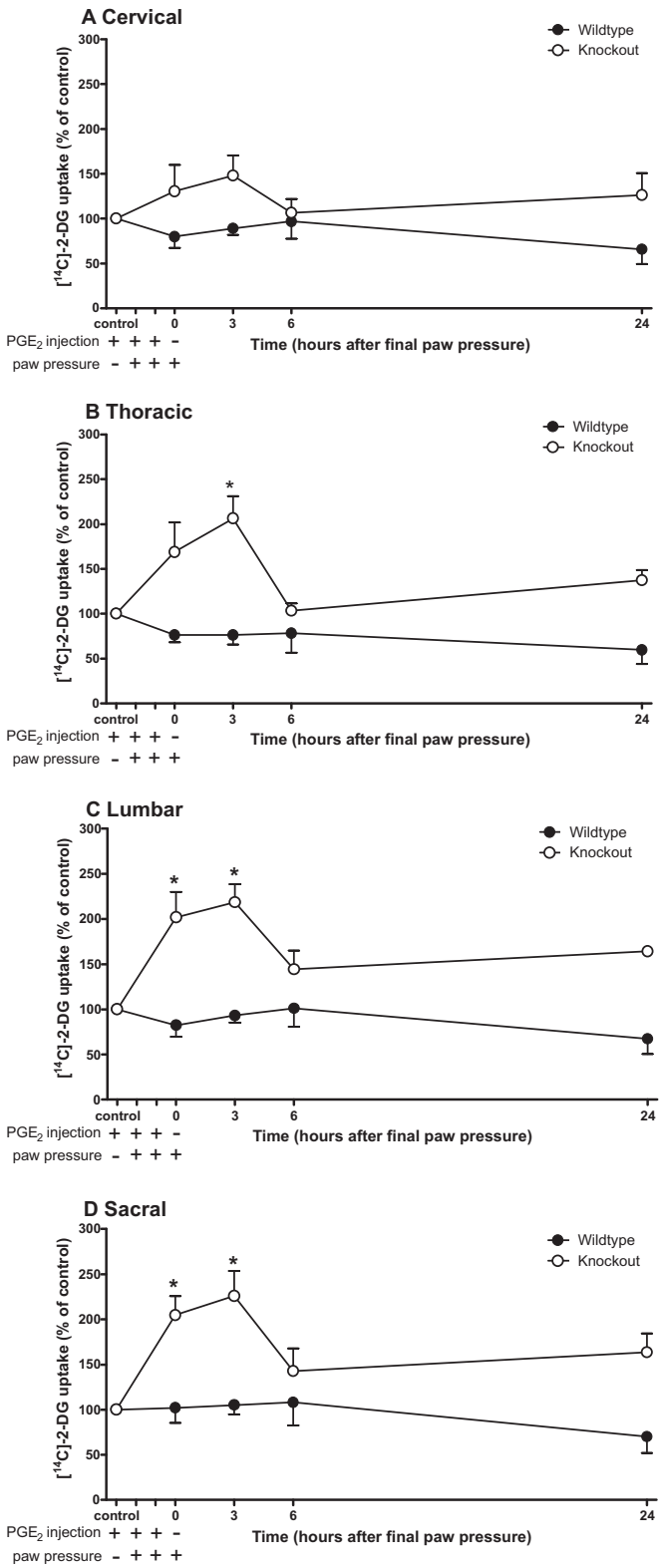

Fig. 2. Uptake of [^{14}C]-2-deoxyglucose ([^{14}C]-2-DG) into spinal cord sections of wildtype and adenosine A_{2A} receptor knockout mice after repeated PGE_2 injection and mechanical paw pressure (mean ± S.E.M., n = 3–7). Measurements were taken from (A) cervical, (B) thoracic, (C) lumbar and (D) sacral regions, and expressed as a percentage of the values before the first injection of PGE_2. Two-way ANOVA revealed a significant genotype effect in lumbar and sacral regions ($P < 0.05$). *$P < 0.05$, time point vs control. One-way ANOVA followed by Dunnett's post hoc test on untransformed data to reveal time differences.

anti-inflammatory effect [5]. When [^{14}C]-2-deoxyglucose was used as a marker for neuronal activity, there was no change in the wild-type mice but an increase in the lumbar and sacral regions both immediately after the end of the stimulus and persisting for 3 h in the A_{2A} knockout mice. This increase in [^{14}C]deoxyglucose uptake cannot be attributed solely to activation of sensory nerves but reflects increased metabolic activity within the spinal cord, which may include activation of descending pathways. Indeed, it may not simply be due to an increase in neuronal firing but may also reflect increased metabolic activity in other cell types, or long-term effects such as increased protein synthesis. However, as it was triggered by the PGE_2/paw pressure treatment it is likely to be a consequence of the inflammatory stimulus applied. Again, the greater response in the A_{2A} knockout mice is likely to reflect the anti-inflammatory role of the A_{2A} receptor, so that the genetic loss of this receptor enhances spinal cord activity in response to a prolonged inflammatory stimulus rather than decreasing it as in the formalin test. For both the [^{3}H]MK801 binding and the [^{14}C]-2-deoxyglucose uptake the changes were more marked in the lumbar and sacral regions than higher up the spinal cord, possibly because the PGE_2 was injected into the hind paw.

These results are in contrast to the recently published report showing that the hyperalgesic response to injection of carrageenan, was reduced in A_{2A} knockout mice [23]. In this study local injection of the A_{2A} antagonist ZM241385 also reduced hyperalgesic responses, but only in female mice. Although carrageenan does cause inflammation and in this study there was an increase in paw oedema in both genotypes, the primary mechanism of the hyperalgesic response to carageenan is chemical stimulation of primary afferents [27]. The reduction in response to carageenan seen in the A_{2A} knockout mice may therefore reflect reduced sensory input due to loss of stimulatory receptors on the primary afferents. We found in the formalin test that the time spent licking or biting the injected paw was only significantly reduced by knockout of the A_{2A} receptor in the first phase of the response, which is largely due to direct stimulation of primary afferents, whereas there was no significant reduction in the second phase which has an inflammatory component [14]. When we measured flinches there was a significant reduction in both phases in the A_{2A} knockout mice, but it was more marked in the first phase, This again suggests that whereas A_{2A} receptor knockout reduces the activation of primary afferents it does not reduce the response to inflammation so effectively.

5. Conclusions

Overall, our previous results using [^{3}H]MK801 binding and [^{14}C]-2-deoxyglucose uptake after formalin treatment, a short nociceptive stimulus, show reduced changes in the spinal cord of A_{2A} knockout mice [15]. This is in agreement with the hypothesis that A_{2A} receptors on peripheral nerves enhance nociceptive input into the spinal cord, and that this is the likely explanation for the antinociceptive effects of A_{2A} antagonists and the reduced nociceptive responses seen in A_{2A} knockout mice [4,12,14,22]. However, the enhanced neurochemical changes seen in the spinal cord of A_{2A} knockout mice after PGE_2/paw pressure, a more prolonged inflammatory stimulus, implies that in this situation the loss of the A_{2A} receptor on inflammatory cells outweighs the loss on peripheral neurones. The relevance and importance of this finding is that although A_{2A} antagonists have antinociceptive effects, they may have only limited use as analgesics in chronic inflammatory pain.

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References

[1] K.O. Aley, J.D. Levine, Role of protein kinase A in the maintenance of inflammatory pain, J. Neurosci. 19 (1999) 2181–2186.
[2] K.O. Aley, G. McCarter, J.D. Levine, Nitric oxide signaling in pain and nociceptor sensitization in the rat, J. Neurosci. 18 (1998) 7008–7014.
[3] K.O. Aley, R.O. Messing, D. Mochly-Rosen, J.D. Levine, Chronic hypersensitivity for inflammatory nociceptor sensitization mediated by the epsilon isozyme of protein kinase C, J. Neurosci. 20 (2000) 4680–4685.
[4] A. Bailey, C. Ledent, M. Kelly, S.M.O. Hourani, I. Kitchen, Changes in spinal δ and κ opioid systems in mice deficient in the A_{2A} receptor gene, J. Neurosci. 22 (2002) 9210–9220.
[5] M.J.L. Bours, E.L.R. Swennen, F. Di Virgilio, B.N. Cronstein, P.C. Dagnelie, Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation, Pharm. Ther. 112 (2006) 358–404.
[6] R.F. Claudino, C.A.L. Kassuya, J. Ferreira, J.B. Calixto, Pharmacological and molecular characterization of the mechanisms involved in prostaglandin E_2-induced mouse paw edema, J. Pharmacol. Exp. Ther. 318 (2006) 611–618.
[7] A.H. Dickenson, Spinal cord pharmacology of pain, Br. J. Anaesth. 75 (1995) 193–200.
[8] R. Dingledine, K. Borges, D. Bowie, S.F. Traynelis, The glutamate receptor ion channels, Pharmacol. Rev. 51 (1999) 7–61.
[9] B.B. Fredholm, Adenosine receptors as drug targets, Exp. Cell Res. 16 (2010) 1284–1388.
[10] B.B. Fredholm, A.P. Ijzerman, K.A. Jacobson, K.N. Klotz, J. Linden, International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors, Pharmacol. Rev. 53 (2001) 527–552.
[11] B.B. Fredholm, A.P. Ijzerman, K.A. Jacobson, J. Linden, C.E. Muller, International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors – an update, Pharmacol. Rev. 63 (2011) 1–34.
[12] L. Godfrey, L. Yan, G.D. Clarke, C. Ledent, I. Kitchen, S.M.O. Hourani, Modulation of paracetamol antinociception by caffeine and by selective adenosine A_2 receptor antagonists in mice, Eur. J. Pharmacol. 531 (2006) 80–86.
[13] A.N. Hata, R.M. Breyer, Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation, Pharm. Ther. 103 (2004) 147–166.
[14] M.J. Hussey, G.D. Clarke, C. Ledent, S.M.O. Hourani, I. Kitchen, Reduced response to the formalin test and lowered spinal NMDA glutamate receptor binding in adenosine A_{2A} receptor knockout mice, Pain 129 (2007) 287–294.
[15] M.J. Hussey, G.D. Clarke, C. Ledent, I. Kitchen, S.M.O. Hourani, Genetic deletion of the adenosine A_{2A} receptor reduces the changes in spinal cord NMDA receptor binding and glucose uptake caused by a nociceptive stimulus, Neurosci. Lett. 479 (2010) 297–301.
[16] B. Johansson, L. Halldner, T.V. Dunwiddie, S.A. Masino, W. Poelchen, L. Gimenez-Llort, R.M. Escorihuela, A. Fernandez-Teruel, Z. Wiesenfeld-Hallin, X.J. Xu, A. Hardemark, C. Betsholtz, E. Herlenius, B.B. Fredholm, Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 9407–9412.
[17] A. Kaelin-Lang, T. Lauterburg, J.M. Burgunder, Expression of adenosine A_{2A} receptor gene in rat dorsal root and autonomic ganglia, Neurosci. Lett. 246 (1998) 21–24.
[18] A. Kaelin-Lang, T. Lauterburg, J.M. Burgunder, Expression of adenosine A_{2A} receptors gene in the olfactory bulb and spinal cord of rat and mouse, Neurosci. Lett. 261 (1999) 189–191.
[19] C.A.L. Kassuya, J. Ferreira, R.F. Claudino, J.B. Calixto, Intraplantar PGE_2 causes nociceptive behaviour and mechanical allodynia: the role of prostanoid receptors and protein kinases, Br. J. Pharmacol. 150 (2007) 727–737.
[20] S.G. Khasar, J.F. Wang, Y.O. Taiwo, P.H. Heller, P.G. Green, J.D. Levine, Mu-opioid agonist enhancement of prostaglandin-induced hyperalgesia in the rat: a G-protein beta gamma subunit-mediated effect? Neuroscience 67 (1995) 189–195.
[21] I. Kitchen, Modification of an analgesy meter for paw-pressure antinociceptive testing in neonatal rats, J. Pharmacol. Methods 12 (1984) 255–258.
[22] C. Ledent, J. Vaugeois, S.N. Schiffman, T. Pedrazzini, M. El Yacoubi, J. Vanderhaeghen, J. Costentin, J.K. Heath, G. Vassart, M. Parmentier, Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A_{2A} receptor, Nature 388 (1997) 674–678.
[23] L. Li, J.X. Hao, B.B. Fredholm, G. Schulte, Z. Weisenfeld-Hallin, X.J. Xu, Peripheral adenosine A_{2A} receptors are involved in carrageenan-induced mechanical hyperalgesia in mice, Neuroscience 170 (2010) 923–928.
[24] J.M. McCoy, J.R. Wicks, L.P. Audoly, The role of prostaglandin E_2 receptors in the pathogenesis of rheumatoid arthritis, J. Clin. Invest. 110 (2002) 651–658.
[25] M.J. Millan, The induction of pain: an integrative review, Prog. Neurobiol. 57 (1999) 1–164.
[26] R.M. Rezende, P. Paiva-Lima, W.G.P. Dos Reis, V.M. Camelo, Y.S. NBakhle, J.N. Francischi, Celecoxib induces tolerance in a model of peripheral inflammatory pain, Neuropharmacol. 59 (2010) 551–557.
[27] J. Sandkuhler, Models and mechanisms of hyperalgesia and allodynia, Physiol. Rev. 89 (2009) 707–758.
[28] J. Sawynock, X.J. Liu, Adenosine in the spinal cord and periphery, Prog. Neurobiol. 69 (2003) 313–340.
[29] J. Sawynok, Adenosine receptor activation and nociception, Eur. J. Pharmacol. 317 (1998) 1–11.