

## Consequences of Eliminating Adenosine A<sub>1</sub> Receptors in Mice

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**ABSTRACT** The second coding exon of the adenosine A<sub>1</sub> receptor gene was eliminated by homologous recombination. The phenotype of mice (mixed C57B6/129OlaHsd background) was studied, using siblings from matings of heterozygous mice. Among the offspring the ratio between +/+, +/- and -/- animals was 1:2:1. Over the first half-year—at least—growth and viability were the same in all genotypes. Binding of A<sub>1</sub> ligands was eliminated in -/- mice and halved in +/- mice. Blood pressure was increased in -/- mice and this was paralleled by an increase in plasma renin. Heart rate was unaffected, as

Contract grant sponsor: Swedish Science Research Council; Contract grant numbers: 2553, 3552, 12587; Contract grant sponsor: The European Commission; Contract grant numbers: FIS 99/1230, QLRT 2000-00069; Contract grant sponsors: Bergvalls Foundation, the Swedish Foundation for Strategic Research, the Wallenberg Foundation, the Swedish Heart and Lung Foundation; Contract grant sponsor: the Danish Medical Research Council; Contract grant number: 9902742; Contract grant sponsors: Veterans Administration, National Institutes of Health, the Bank of Sweden Tercentenary Foundation.

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†Tom Dunwiddie died in a climbing accident in 2001. This article is dedicated to his memory.

Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/ddr.10170

was contractility. Furthermore, the response of the perfused heart to ischemia was similar in +/+ and -/- hearts. However, remote preconditioning was eliminated in -/- mouse hearts. Tubuloglomerular feedback in the kidney was also lost in -/- mice. The analgesic response to a non-selective adenosine receptor agonist was lost in -/- mice, which also showed hyperalgesia in the tail-flick test. There was a slight hypoactivity in -/- mice, but responses to caffeine were essentially normal. The inhibition of excitatory neurotransmission in hippocampus by adenosine was lost in -/- mice and reduced in +/- mice. Responses to ATP were affected similarly. Hypoxic depression of synaptic transmission was essentially eliminated in hippocampus and hypoxic decrease in spinal respiratory neuron firing was markedly reduced. These results show that adenosine A<sub>1</sub> receptors play a physiologically important role in the kidney, spinal cord, and hippocampus and that they are critically important in the adaptive responses to hypoxia. *Drug Dev. Res.* 58:350–353, 2003. © 2003 Wiley-Liss, Inc.

**Key words:** adenosine A<sub>1</sub> receptor gene; mice; hypoxia; pain; anxiety

## INTRODUCTION

Even though quite selective agonists and antagonists are available for adenosine A<sub>1</sub> receptors [Fredholm et al., 2001], and consequently much is known about their role in physiology and pathophysiology, we embarked on a classical gene knock-out strategy. The second coding exon of the A1R gene was targeted as described [Johansson et al., 2001] and experiments were conducted on littermates from matings of heterozygote animals (A1R+/-) with a mixed C57Bl6/129OlaHsd background.

The offspring showed the expected frequency (1/4 A1R+/+, 2/4 A1R+/- and 1/4 A1R-/-). There were no clear differences in the growth and maturation over the first 5 months between genotypes [Johansson et al., 2001], but subsequently +/- and, especially, -/- mice tended to die earlier than their +/- littermates [Giménez-Llort et al., 2002]. So far we have not observed any major differences in fertility between genotypes, even though there is some evidence that the A<sub>1</sub> receptor is involved in sperm capacitation [Minelli et al., 1995; Minelli, personal communication].

## BEHAVIOR

A1R+/- and -/- mice showed no gross behavioral abnormalities. However, their grip strength, as judged by the so-called wire hang test, was reduced [Giménez-Llort et al., 2002]. This was somewhat surprising given the evidence that methylxanthines in doses that predominantly affect adenosine receptors appear, if anything, to increase muscle strength. However, recent *in vitro* studies [Reading and Barclay, 2001] show that A<sub>1</sub> receptor activation improves skeletal muscle function.

Although overall locomotor activity was unaltered in A1R-/- mice [Johansson et al., 2001], we have found that the increase in motor activity that accom-

panies transition from light to dark is reduced in the knock-out mice [Giménez-Llort et al., 2002]. Surprisingly, A1R+/- mice were in this regard indistinguishable from wild-type animals. However, exploratory activity (assayed in the open field and the hole-board tests) tended to be increased in the heterozygotes [Giménez-Llort et al., 2002]. This is consistent with the known stimulatory effect of methylxanthines, including caffeine [Fredholm et al., 1999].

The A1R-/- mice showed increased anxiety in two commonly used tests, the elevated plus maze and the dark-light box [Giménez-Llort et al., 2002]. This is interesting because hyperanxiety was also observed in A2A-/- mice [Ledent et al., 1997]. Future experiments will tell whether the traits in the two strains are synergistic. It is also interesting to compare with the fact that high doses of caffeine, that acts on A<sub>1</sub> and A<sub>2A</sub> receptors, produce anxiety in animals and humans [Fredholm et al., 1999]. In the resident intruder test A1R-/- mice also exhibited increased aggressiveness [Giménez-Llort et al., 2002], again a trait also observed in A2A-/- mice [Ledent et al., 1997]. By contrast, no effect was observed when examining memory functions with the Morris water maze.

## NEURONAL ACTIVITY

Using the hippocampal slice preparation, the inhibitory adenosine effects were shown to be eliminated in A1R-/- mice [Johansson et al., 2001]. This is an important finding because it shows that there are no important effects mediated via any of the other receptors in this preparation, despite the fact that important effects of both A<sub>2A</sub> [Sebastiao and Ribeiro, 1996] and A<sub>3</sub> receptors [Dunwiddie et al., 1997] have been reported. Another important finding was that the dose-response curve for adenosine was shifted significantly (twofold) to the left in A1R+/- mice, which possess almost exactly half the normal

number of receptors. This emphasizes the important general point that potency of adenosine analogues is strongly dependent on the number of receptors. There were no adaptive changes in responses to GABA<sub>B</sub> receptor activation or any adaptive changes in A<sub>2A</sub> receptors.

### ALTERED RESPONSE TO HYPOXIA

Ever since the pioneering work of Berne and Gerlach, a role for adenosine in mediating responses to hypoxia and ischemia has been postulated. We have so far examined the role of A<sub>1</sub> receptors in two such responses. In the hippocampal slice preparation, responses to hypoxia include a fast decrease in responses to excitatory stimulation. We found that virtually all of this decrease was lost in slices from A1R<sup>-/-</sup> mice [Johansson et al., 2001]. Furthermore, A1R<sup>-/-</sup> slices did not fully recover after hypoxia, as did A1R<sup>+/+</sup> slices. These findings clearly demonstrate that adenosine acting on A<sub>1</sub> receptors is critically important in regulating the responsiveness of neurons to decreased supply of metabolizable energy, enabling the neurons to survive such energy shortage.

A somewhat similar effect was observed when activity of respiratory neurons in the immature brainstem was studied. Much of the normal depression of rate of spontaneous firing induced by hypoxia was lost or markedly reduced in A1R<sup>-/-</sup> mice. Also in this preparation, the recovery seen in A1R<sup>+/+</sup> mice was less complete in A1R<sup>-/-</sup> brainstem neurons [Johansson et al., 2001].

Body temperature was similar in all genotypes, but the temperature-lowering effect of adenosine analogues was less pronounced in A1R<sup>+/+</sup> and, in particular, in A1R<sup>-/-</sup> mice [Johansson et al., 2001].

### HYPERALGESIA

It has long been known that adenosine can control pain [Geiger et al., 1984; Sawynok and Sweeney, 1989; Sosnowski and Yaksh, 1989] and adenosine receptors, particularly A<sub>1</sub> receptors, have been known to be present in spinal cord [Geiger et al., 1984; Fastbom et al., 1990]. It was therefore no major surprise when we found that the analgesic effects of intrathecal adenosine analogues were essentially lost in A1R<sup>-/-</sup> mice [Johansson et al., 2001]. Somewhat more surprising was the finding that there was a significant hyperalgesia in these animals. This clearly suggests that adenosine acting at A<sub>1</sub> receptors constitutes a significant endogenous analgesic mechanism.

The actions of adenosine, however, are also mediated by other adenosine receptors. Thus, adenosine A<sub>2A</sub> receptors, presumably located at sensory nerve endings, mediate hyperalgesia [Ledent et al.,

1997]. A<sub>3</sub> receptors are—at least in mice—important as promoters of peripheral inflammation that leads to pain [Wu et al., 2002].

### CARDIOVASCULAR EFFECTS

It has long been known that adenosine can decrease cardiac performance and reduce heart rate [Drury and Szent-György, 1929], and this effect, which is clinically relevant, is linked to A<sub>1</sub> receptors [Lerman and Belardinelli, 1991]. The depressant effect of exogenous adenosine analogues was virtually eliminated in A1R<sup>-/-</sup> mice. It was therefore surprising that heart rate in A1R<sup>-/-</sup> mice proved to be similar to that in their wild-type littermates.

Blood pressure in anesthetized mice was elevated in the A1R<sup>-/-</sup> genotype (12 mm Hg), and there was even a tendency towards an elevated blood pressure in A1R<sup>+/+</sup> mice (5 mm Hg) [Brown et al., 2001]. Because A1R<sup>-/-</sup> mice also had an elevated level of plasma renin, a possible explanation is that the blood pressure elevation is due to angiotensin. However, an angiotensin receptor antagonist had similar blood pressure lowering effect between genotypes [Brown et al., 2001]. There were no major differences in blood vessel reactivity between genotypes, so we are still looking for the cause of the blood pressure increase. Given that the A2AR<sup>-/-</sup> genotype is also hypertensive [Ledent et al., 1997], it will be interesting to know if the double knock-out shows a marked blood pressure elevation.

In preliminary studies, we have found that isolated hearts from A1R<sup>-/-</sup> mice do not differ in their response to acute ischemia from hearts from their wild-type littermates. However, we do see a markedly reduced protective effect of remote preconditioning. Ongoing studies focus on demonstrating the underlying mechanisms.

### RENAL EFFECTS

Sodium excretion was markedly (twofold) elevated in both A1R<sup>+/+</sup> and <sup>-/-</sup> mouse kidneys [Brown et al., 2001]. This agrees with the known effects of caffeine and other adenosine receptor antagonists. By contrast, potassium excretion and glomerular filtration rate was unaltered. Most importantly, tubuloglomerular feedback is completely eliminated in A1R<sup>-/-</sup> mice [Brown et al., 2001]. These results emphasize that renal A<sub>1</sub> receptors constitute important drug targets.

### USE IN DRUG SELECTIVITY STUDIES

Knock-out mice can be used not only to determine the role(s) of a particular gene product in physiology and pathophysiology, but also to determine the selectivity of drugs. So far we have finished a study that demonstrates that at least some of the effects of

ATP (and other adenine nucleotides) in the hippocampus are in fact mediated by A<sub>1</sub> receptors, because the responses are completely eliminated in A1R<sup>-/-</sup> mouse hippocampi [Masino et al., 2002]. It will be important in the future to perform similar studies in other tissues, because adenine nucleotides are so rapidly broken down at cell membranes, generating high local concentrations of adenosine.

### CONCLUSIONS

The major conclusion, so far, from the studies of the phenotype of A<sub>1</sub> receptor knock-out mice is that these mice show a surprisingly normal physiology. It is only in situations (hypoxia, pain, increased kidney work, cardiac ischemia) where the organism is strongly perturbed that effects are seen. This suggests that drugs targeting A<sub>1</sub> receptors might not have too many side effects under basal conditions. The results also emphasize that the role(s) of adenosine may be particularly important pathophysiologically.

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