Knockout Corner: Neurobehavioural consequences of a serotonin 5-HT$_{2C}$ receptor gene mutation

Lora K. Heisler and Laurence H. Tecott
Department of Psychiatry, University of California at San Francisco, San Francisco, CA 94143

Abstract
Studies employing nonselective serotonin 5-HT$_{2C}$ receptor agonists and antagonists have implicated this receptor subtype in many of the actions of serotonin. To further examine the function of this receptor, 5-HT$_{2C}$ receptor mutant mice were generated; studies of these animals reveal pleiotropic neurobehavioural effects of the mutation. Three examples are described: (1) Mutants exhibit chronically elevated food intake and the development of an obesity syndrome during the ’middle-age’ portion of their lifespan. Their potential utility as a model of human obesity is further indicated by their enhanced sensitivity to high-fat feeding, leading to the development of type 2 diabetes. (2) 5-HT$_{2C}$ receptor mutants also display infrequent and sporadic spontaneous seizures. Further studies suggested the presence of globally enhanced neuronal network excitability in these mice. These findings raise the possibility that 5-HT$_{2C}$ receptors mediate a role for serotonin in the suppression of seizure activity. (3) Behavioural analysis of mutant mice revealed abnormal performance in a spatial learning task and altered exploratory behaviour, associated with perturbed long-term potentiation restricted to the dentate gyrus perforant path synapse. Taken together, the above findings implicate 5-HT$_{2C}$ receptors in the serotonergic regulation of feeding, neuronal network excitability, and hippocampal function.

Received 6 December 1998; Reviewed 31 December 1998; Revised 31 December 1998; Accepted 31 December 1998

Key words: Serotonin, obesity, epilepsy, spatial learning, dentate gyrus.

Introduction
Serotonergic neurons innervate all levels of the neuraxis and regulate diverse physiological and behavioural processes. In recent years, at least 14 distinct serotonin receptor subtypes have been identified and their genes have been molecularly cloned (Peroutka, 1994). The relative contribution of these receptor subtypes to the actions of serotonin has been difficult to determine due to the limited availability of receptor subtype selective pharmacological agents. A complementary strategy to investigate serotonin receptor subtype function is to develop animals with targeted mutations of the genes encoding these receptors. Molecular genetic and pharmacological approaches complement each other, clarifying the physiological significance of individual receptor subtypes.

For these reasons, a gene targeting approach has been used to examine the functional roles of the 5-HT$_{2C}$ receptor (Tecott et al., 1995). This receptor subtype has been implicated in many actions of serotonin. In humans and in rodent models, nonselective 5-HT$_{2C}$ receptor agonists, such as 1-(3-chlorophenyl)piperazine (m-CPP), produce anxiogenic effects and decrease feeding, actions that are blocked by 5-HT$_{2C}$ receptor antagonists (e.g. Curzon et al., 1997; Gibson et al., 1994). However, a lack of 5-HT$_{2C}$ receptor selective agonist, and antagonist compounds has complicated the interpretation of pharmacological studies. Additional clues to the functions of this receptor are provided by the multiple neurobehavioural perturbations observed in 5-HT$_{2C}$ receptor mutant mice.

‘Middle-aged’ obesity and type 2 diabetes
Supporting an important role for the 5-HT$_{2C}$ receptor in the serotonergic regulation of feeding, an obesity syndrome was observed in mutant mice (Tecott et al., 1995). These animals exhibit a 25–30% increase in food intake, beginning by 2 months of age and persisting throughout the life of the animal. Interestingly, obesity does not develop until approx. 5–6 months of age, the ’middle-
marked age-dependent physiological changes in the mutants were further indicated by examination of glucose homeostasis and responsiveness to the adipocyte hormone leptin. Young adult mutant mice displayed normal levels of plasma insulin and normal responsiveness to the effects of leptin treatment. By contrast, older obese mutant mice exhibited elevations in plasma levels of insulin and reduced glucose tolerance, reflecting the development of insulin resistance. These results indicate that the 5-HT<sub>2c</sub> receptor mutant does not directly alter insulin signalling. Rather, age-related changes in these systems are more likely to represent indirect consequences of chronic hyperphagia.

As dietary fat intake plays an important role in the development of both obesity and type 2 diabetes, the sensitivity of mutants to a high fat diet was also examined. High fat feeding produced rapid weight gain in young adult mutant mice, leading to a premature divergence of body weight from wild-type levels. In addition, mutants fed the high fat diet rapidly developed hyperinsulinaemia and had plasma glucose levels in the diabetic range. These findings indicate that a genetic alteration of brain serotonin signalling can predispose to type 2 diabetes mellitus.

In contrast to other animal genetic models of obesity, such as the ob/ob and db/db strains, the hyperphagia of the 5-HT<sub>2c</sub> receptor mutants clearly precedes alterations of insulin and leptin signalling. Thus, the 5-HT<sub>2c</sub> receptor mutation is likely to produce a primary feeding dysregulation, leading to overconsumption, rather than a primary increase in energy efficiency. An age-dependent inability to compensate for this hyperphagia causes enhanced fat deposition, and the observed endocrine perturbations may be secondary to the obese state. Several features of the obesity syndrome of 5-HT<sub>2c</sub> receptor mutant mice are reminiscent of common forms of human obesity. These include ‘middle-age’ onset, insulin resistance, hyperleptinaemia and enhanced susceptibility to type 2 diabetes.

The abundance of evidence implicating 5-HT<sub>2c</sub> receptors in the regulation of feeding raises the possibility that these receptors contribute to the anorectic properties of nonselective serotonergic agonists, such as m-CPP, and the appetite suppressant dexfenfluramine. To test this hypothesis, the sensitivity of 5-HT<sub>2c</sub> receptor mutants to these drugs was examined. The anorectic effects of m-CPP were eliminated and those of dexfenfluramine substantially reduced in mutant mice (Tecott et al., 1995; Vickers et al., In Press). These results indicate that 5-HT<sub>2c</sub> receptors contribute to the anorectic effect of these compounds and that this receptor subtype may be a reasonable target for the development of new appetite-suppressant drugs.

### Elevated neuronal network excitability

Videotape monitoring of mutant mice revealed the presence of a seizure syndrome, manifested by spontaneous tonic-clonic seizures associated with the enhancement of grooming behaviours. Spontaneous seizures were found to be infrequent and sporadic in 5-HT<sub>2c</sub> receptor mutant mice. To gain a more quantitative measure of the extent to which the mutation had altered seizure susceptibility, the sensitivity of animals to i.v. administration of the convulsive drug pentamethylenetetrazole (metrazol) was determined. Mutant mice exhibited a 24% reduction in seizure threshold (latency or cumulative dose required for the first twitch) and an 83% reduction in the duration of the tonic–clonic phase of the response relative to wild-type animals. These results suggest that the loss of 5-HT<sub>2c</sub> receptors leads to both a lowered seizure threshold and a more rapid progression of seizure activity. This interpretation is supported by the finding that the nonspecific 5-HT<sub>2c</sub> receptor antagonist mesulergine enhanced metrazol sensitivity in wild-type mice.

Analysis of spontaneous seizures in 5-HT<sub>2c</sub> receptor mutant mice is difficult, due to their rarity. We therefore tested whether seizures could be induced in mutants by a noninvasive sound stimulus (Brennan et al., 1997). Mutants exposed to a complex acoustic stimulus consisting of a mixture of high frequency tones displayed behavioural evidence of seizures within seconds of sound presentation. Shortly after tone onset, the mutants exhibited the sudden onset of a bout of wild running and erratic leaping lasting 1–2 s, followed by a period of extensor rigidity. To characterize audiogenic seizure pathways within the brain, immunocytochemistry was performed for c-fos, a marker of neuronal activity. Following seizures, staining was observed in subcortical structures associated with auditory processing in a pattern similar to those found in other AGS-susceptible strains. This epilepsy syndrome represented the first audiogenic seizure model for which the causative genetic perturbation has been identified. Subsequent studies revealed enhanced sensitivity of mutants to a variety of convulsant stimuli, such as electroshock, electrical kindling of the olfactory bulb, and the chemoconvulsant flurothyl. These results reveal a global enhancement of seizure susceptibility in 5-HT<sub>2c</sub> receptor mutant mice (Applegate and Tecott, 1998).
Taken together the above findings are consistent with several lines of evidence indicating an anticonvulsant role for serotonin. Due to the limited selectivity of pharmacological tools, the identity(ies) of the receptor subtypes contributing to these actions has been unclear. These results strongly implicate 5-HT$_{2C}$ receptors in the serotonergic inhibition of neuronal network excitability, and indicate a potential direction for anticonvulsant drug development. Interestingly, several commonly used psychiatric drugs with potent 5-HT$_{2C}$ receptor antagonist properties lower seizure thresholds and promote weight gain (Jenck et al., 1994). 5-HT$_{2C}$ receptor antagonism may therefore contribute to these troubling side effects.

**Perturbed hippocampal function**

The hippocampal formation is believed to mediate cognitive processes such as learning and memory. Because 5-HT$_{2C}$ receptors are abundantly expressed throughout the hippocampal formation, hippocampal-dependent behaviours, such as spatial learning, were examined in 5-HT$_{2C}$ receptor mutant mice. These animals displayed abnormal performance in a spatial learning task. Interestingly, the abnormal learning performance of 5-HT$_{2C}$ receptor mutants did not generalize to all forms of learning, as indicated by normal contextual discrimination (Tecott et al., 1998) and normal acquisition of operant responding for food (Dr Lisa Gold, personal communication).

To determine whether this behavioural abnormality was associated with alterations in hippocampal physiology, long-term potentiation (LTP), a phenomenon thought to model learning-related neural plasticity, was assessed in mutant mice. When LTP was measured at multiple hippocampal subregions, a reduction was observed at the perforant path dentate gyrus synapse though not at other sites. These results indicate that the 5-HT$_{2C}$ receptors may contribute to the serotonergic modulation of this hippocampal structure and that plasticity in this region may influence spatial learning.

**Conclusions**

The pleiotropic effects of the 5-HT$_{2C}$ receptor mutation reveal the diverse processes that may be influenced by a single gene product. This work also illustrates the use of targeted receptor gene mutations to uncover new roles for receptor subtypes. The clinical relevance of 5-HT$_{2C}$ receptor mutant mice is highlighted by their utility as a model of ‘middle-aged’ obesity and epilepsy. These animals, and mice bearing mutations of other receptor genes, will be useful for clarifying the contribution of individual receptor subtypes to both the therapeutic and adverse actions of psychiatric drugs, leading to the development of more effective treatments for psychiatric disorders.

**References**