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4 Crestani, F. *et al.* (2000) Resolving differences in $GABA_A$ receptor mutant mouse studies. *Nat. Neurosci.* 3, 1059
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Chemical name

L838417: 7-tert-butyl-3-(2,5-difluoro-phenyl)-6-(2-methyl-2*H*-[1,2,4]triazol-3-ylmethoxy)-[1,2,4]triazolo[4,3-*b*]pyridazine

Anxiolytic-like action of diazepam: mediated by $GABA_A$ receptors containing the α2-subunit

Response from Crestani *et al.*

Using a point-mutation strategy the anxiolytic-like effect of diazepam was recently attributed to $GABA_A$ receptors that contain the α2-subunit[1]. This finding was based on the lack of an anxiolytic-like response to diazepam of α2(H101R) mice but not of α3(H126R) mice in the elevated plus maze (EPM) and the light–dark box (LDB) tests. Because these tests involve locomotion, a direct drug-induced motor effect has to be excluded.

In their study on α1(H101R) mice, McKernan and colleagues showed that diazepam increased locomotor activity in α1(H101R) mice[2]. In addition, in wild-type mice, diazepam was without effect on locomotor activity[2]. The test conditions of McKernan and colleagues included a transfer of the animals to an unfamiliar environment for testing, thereby subjecting them to a stressful experience[2]. We obtained similar results in α1(H101R) mice when we likewise transferred the animals to an unfamiliar testing room before the test (30 min before drug treatment)[3]. By contrast, under our standard test conditions, mice are kept in the testing room for at least 14 days before testing. In a familiar environment, diazepam did not stimulate motor activity in α1(H101R) mice and displayed sedative action in wild-type mice[4]. In the LDB and the EPM tests themselves, no motor deficits were observed either in α2(H101R) mice[1] or in α3(H126R) mice. In the LDB, both the number of entries into the dark area (from the tunnel) and the time spent in the dark area remained unaffected by diazepam treatment in wild-type, α2(H101R) and α3(H126R) mutant mice. Similarly, in the EPM, the number of enclosed arm entries and the time spent in the enclosed arms were not altered by diazepam treatment. The mean number of entries into the enclosed arms is an estimate of protected exploration and locomotor activity. The argument made by Reynolds and colleagues: 'If the α2-subtype mediates the stimulatory effects then... Similarly, if the α3-subtype mediates the stimulatory effects then...' is therefore purely hypothetical and irrelevant in the context of our experiments[1]. There is no experimental evidence to indicate that diazepam would induce a motor hypoactivity in α2(H101R) or α3(H126R) mice under our test conditions in the LDB and the EPM tests. In addition, it is noteworthy that the α2(H101R) mice retained an anxiolytic-like response to sodium phenobarbital in the LDB (Ref. 1).

Despite their own concerns (see above), McKernan *et al.*[2] made use of the EPM test to determine the anxiolytic-like activity of L838417 in rats. This ligand has an agonistic activity at α2-, α3- and α5-containing $GABA_A$ receptors but not at α1-containing $GABA_A$ receptors and would be comparable in its action on locomotion to that of diazepam in α1(H101R) mice. In their study[2], McKernan and colleagues did not state whether the locomotor activity *per se* was altered by the drug.

In summary, when interpreting results from behavioural studies, the environmental and technical experimental details must be taken into account. A diazepam-induced enhancement of locomotion in α1(H101R) mice appears to be stress related. Under our experimental conditions of the LDB and EPM tests, diazepam did not display a stimulatory effect on locomotion. The argument put forward by Reynolds and colleagues therefore does not warrant an interpretation of the data different from that published previously[1,4,5].

Florence Crestani
Institute for Pharmacology and Toxicology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.
Hanns Möhler
Institute for Pharmacology and Toxicology, University of Zürich and Dept of Applied Biosciences, Swiss Federal Institute of Technology (ETH) Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.
Uwe Rudolph*
Institute for Pharmacology and Toxicology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.
*e-mail: rudolph@pharma.unizh.ch

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