Factors influencing the disappearance of radioactivity from mouse brain after injections of tracer doses of the very selective receptor antagonists [3H]raclopride and [3H]robalzotan (NAD-299) which bind with high affinity to dopamine-D2 and 5-hydroxytryptamine1A receptors, respectively, were studied. For both ligands the amount of radioactivity in cerebellum was taken as non-specific binding and subtracted from the amount of radioactivity found in the brain regions studied. The disappearance of the radioactivity in naive mice followed apparent first-order reactions with \( T_{1/2} = 16.8 \pm 1.4 \) min for [3H]raclopride in striatum and \( T_{1/2} = 22 \pm 2 \) min and \( 17 \pm 2 \) min for [3H]robalzotan in hippocampus and frontal cortex, respectively. Pretreatment of mice with 1 mg/kg s.c. reserpine 20 h before the experiment strongly prolonged the dissociation phase for the two ligands. Injection of the dopamine-D2 receptor antagonist eticlopride 1 h after [3H]raclopride in the reserpinized mice rapidly reduced the radioactivity in striatum to the same level as in cerebellum. Similarly, the 5-HT1A receptor antagonist WAY-100,635 injected 1 h or 4 h after [3H]robalzotan rapidly reduced the radioactivity in hippocampus and frontal cortex to the cerebellum level showing that the intact drugs were still bound to the receptors. In reserpinized mice kept at 30°C 1 h before and during the experiment, which normalised the body temperature, the disappearance of radioactivity was more like that in untreated animals but was still significantly higher than in the control animals. Mice treated with anaesthetic agents, e.g. \( \gamma \)-butyrolactone (GBL), pentobarbital sodium and chloral hydrate, also strongly prolonged the rate of disappearance of [3H]raclopride and [3H]robalzotan from the receptor-rich brain regions. The pronounced effect of hypothermia on the dissociation of these [3H] ligands from their receptors is probably caused by a general decrease in brain nervous activity. However, the decreased rate of dissociation evoked by reserpine, GBL, baclofen and prazosin after normalisation of the body temperature may be due to specific actions of these compounds causing decrease in dopaminergic and serotonergic nerve activity which results in reduced synaptic concentration of the transmitter amines. In accordance with this view, increased synaptic 5-HT evoked by the 5-HT releasing agent \( p \)-chloroamphetamine enhanced the disappearance of [3H]robalzotan from hippocampus and frontal cortex.

Keywords Raclopride · Robalzotan · Dopamine-D2 receptors · 5-HT1A receptors · Striatum · Hippocampus · Frontal cortex · In vivo binding

Introduction

Raclopride and robalzotan (NAD-299) are very selective antagonists of the dopamine-D2 and 5-hydroxytryptamine1A (5-HT1A) receptors, respectively (Köhler et al. 1985; Johansson et al. 1997). In [3H]-labelled forms they are also useful ligands for in vivo studies of these receptors in the brain (Köhler et al. 1985; Ross and Jackson 1989; Stenfors et al. 1998). As a PET (positron emission tomography) ligand, [11C]raclopride is used not only to measure the occupancies of neuroleptics to dopamine-D2 receptors in the human brain (Farde et al. 1992) but also to record changes in the activities in dopamine neurones (Dewey et al. 1993; Volkow et al. 1994; Breier et al. 1997; Koepp et al. 1998). This is possible because the affinities of raclopride and dopamine for the dopamine-D2 receptor are similar so that these two ligands compete with each other for binding to the dopamine-D2 receptors (Ross and Jackson 1989; Seeman et al. 1989; Young et al. 1991). It should therefore be of great interest to develop PET ligands for other transmitter receptors with properties similar to those of raclopride. Robalzotan may be such a ligand for the serotonergic system.

In a previous study (Stenfors et al. 1998) it was observed that compounds that decrease the firing in serotonergic neurones, e.g. the \( \alpha \)-adrenoceptor antagonist
prazosin and the α₂-adrenoceptor agonist clonidine, increase the in vivo binding of [³H]robalzotan in mouse frontal cortex and hippocampus. This might be explained by reduced synaptic 5-HT competing with robalzotan for binding to the 5-HT₁₅ receptors. Furthermore, the 5-HT releasing compound p-chloroamphetamine decreases the in vivo binding of [³H]robalzotan in mouse brain (unpublished observation).

Because pharmacological manipulation of experimental animals such as the mouse may induce non-specific changes, e.g. hypothermia and cardiovascular effects, we have in the present study examined the effect of hypothermia, induced by different agents, on the in vivo binding of [³H]robalzotan in comparison with that of [³H]raclopride. Part of this work has previously been presented (Stenfors and Ross 1998).

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### Materials and methods

**Subjects.** Male albino mice (NMRI; B&K Universal, Sollentuna, Sweden) weighing 25–32 g were used. They were kept under constant temperature (22°C) and lighting (6 a.m.–6 p.m.) and were allowed free access to food and water. The compounds were injected i.v., i.p. or s.c. in a volume of 0.1 ml/10 g body weight. The animals were killed by cervical dislocation and the brain regions studied were immediately dissected on ice. The study was approved by the local Animal Ethical Committee.

**In vivo binding experiments.** Groups of 4–5 mice were injected in a tail vein with about 2 µCi [³H]raclopride or [³H]robalzotan diluted with unlabelled compounds. This gives a tracer dose between 2 nmol/kg and 3 nmol/kg; the accurate dose was determined from the radioactivity of the injected solution in each experiment. In some experiments the animals were kept at 30°C in a warming cupboard in order to avoid hypothermia during the experiment. The mice were killed at the times indicated for the different experiments. The brain tissues dissected (cerebellum and striatum in the raclopride experiments and cerebellum, hippocampus and frontal cortex in the robalzotan experiments) were stored on an aluminium sheet on ice before they were weighed and dissolved in 0.5 ml Soluene-350 (Packard) at 60°C for 90 min. After addition of 5 ml scintillation liquid (UltimaGold; Packard) the radioactivity was counted in a Beckman LS 8100 liquid scintillation spectrometer using as measures of central tendency and variation, respectively. Analysis of variance (ANOVA) was performed using SYSTAT 7.0: New Statistics (SPSS, USA), followed by the Bonferroni post hoc test. A P-value less than 0.05 was considered significant.

### Results

**Effect of reserpine pre-treatment**

[³H]Raclopride

In agreement with a previous report (Ross and Jackson 1989), [³H]raclopride injected as a tracer dose (3 nmol/kg i.v.) was selectively taken up in dopamine-rich striatum compared with the dopamine-poor cerebellum (Fig. 1A). It disappeared from striatum with a T₁/₂ of 16.8±1.4 min (n=3). In mice pretreated with 1 mg/kg s.c. reserpine 24 h before and kept at room temperature the rate of disappearance of the radioactivity from striatum was very slow with a T₁/₂ value of hours. When the reserpine-treated mice were kept at 32°C 1 h before the [³H]raclopride injection and during the experiment the rate of disappearance of radioactivity from striatum was similar to that in non-treated control animals (Fig. 1A and insert). However, the specific accumulation of radioactivity was significantly higher in the reserpine-treated mice than in the control animals, which is in accordance with previous results by Ross and Jackson (1989). Injection of S-(-)-etioctolpride hydrochloride (0.38 mg/kg s.c.), a selective dopamine-D₂ receptor antagonist (Hall et al. 1985), 60 min after the injection of [³H]raclopride rapidly enhanced the disappearance of radioactivity down to cerebellum levels (Fig. 1B), indicating that the radioactivity in striatum was intact [³H]raclopride bound to the dopamine-D₂ receptor.

[³H]Robarlotanz

In accordance with previous results (Stenfors et al. 1998) the radioactivity in cerebellum disappeared more rapidly than from frontal cortex and hippocampus after injection of a trace dose (2.7 nmol/kg i.v.) of [³H]robalzotan (data not shown). The rate of disappearance followed an apparent first order reaction during the first 15 min in cerebellum with a mean half-life of 8±1 min from three independent experiments. Since the cerebellum concentration represents the non-specific accumulation (binding) of [³H]robalzotan (Stenfors et al. 1998), this was subtracted from the values obtained in frontal cortex and hippocampus giving the specific binding of the ligand to the 5-HT₅ receptors in these regions. The rate of dissociation of the specifically bound [³H]robalzotan from the receptors occurred with a half-life of 17±2 min and 23±2 min in frontal cortex and hippocampus, respectively. In reserpine-treated