COMPARISON OF [^{11}C]CLORGYLINE AND [^{11}C]L-DEPRENYL FOR QUANTIFYING MAO A AND B SUBTYPE ACTIVITIES IN PERIPHERAL ORGANS IN HUMANS

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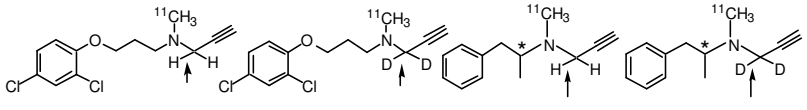
Keywords: monoamine oxidase A and B, peripheral organs

Monoamine oxidase (MAO) is a key regulatory and protective enzyme because its substrates include many physiologically active amines including neurotransmitters, drugs and dietary amines. It occurs in two different subtypes, MAO A and MAO B which are different gene products and have different substrate and inhibitor specificities (reviewed in Shih et al, 1999). The relative ratios of MAO A and B are both organ and species dependent making it difficult to use animals as a model for humans (Inoue et al., 1999). The purpose of this study is to compare the irreversible MAO A and B radiotracers [^{11}C]clorgyline (CLG) and [^{11}C]L-deprenyl (DEP) and their deuterium substituted counterparts (CLG-D and DEP-D) as tracers for measuring MAO A and B in peripheral organs with respect to isotope effect, binding rate, sensitivity to blood flow, and differences in organ distribution. Deuterium substitution was in the methylene carbon of the propargyl group which is the bond known to be cleaved by MAO oxidation.



CLG CLG-D DEP DEP-D

Methods: CLG and CLG-D were imaged for 60 min with PET in 12 healthy human subjects and the results compared with 9 subjects imaged previously with DEP and DEP-D (Fowler et al., 2002). Normalized uptake was measured from the average uptake at plateau (20-60 min) divided by the plasma integral of tracer from injection to end of study (incorporation quotient, *IQ*). A 3-compartment model was used to calculate λk_3, the model term proportional to MAO A or MAO B activity as well as K_1 (plasma to tissue transfer constant), k_2 (tissue to plasma transfer constant) k_3 (kinetic constant controlling the rate of binding to MAO A or B). The ratio $\lambda k_3/K_1$ was used as an estimate of tracer binding to tracer delivery. λ is the ratio K_1/k_2.

Results: (1) Both CLG and DEP showed a robust isotope effect (2.7-5.7; $p<0.001$) on λk_3 in the heart, lungs, kidneys, and spleen which is characteristic of MAO. The magnitude of the isotope effect differed for different organs but not for CLG *vs* DEP. No isotope effect on λk_3 was seen for the liver for either tracer pair. (2) High uptake and a robust isotope effect was seen for the thyroid for CLG but not DEP; (3) The *IQ*'s and λk_3*'s* were consistently higher for DEP in heart, kidneys and spleen but not lung where CLG >DEP; (4) For $\lambda k_3/K_1$, DEP> CLG indicating that DEP binding would be more sensitive to blood flow than CLG. Thus DEP-D is a more sensitive tracer due to its reduced rate of binding while the CLG and CLG-D2 are not as sensitive due to the lower values of $\lambda k_3/K_1$; (5) MAO A but not MAO B is present in the thyroid; (6) MAO A was significantly higher than MAO B in the lung ($p<0.05$); (7) both CLG and DEP have low excretion into the urine (1-3% over 90 min). Summary: The relative regional distribution of MAO A and B in peripheral organs is consistent with post-mortem tissue examination (Saura et al., 1996) with the exception of the liver which we could not measure since it appears to be an excretion pathway for labeled metabolites and cannot be quantified. The high lung MAO A:MAO B ratio is also consistent with post-mortem data (Saura et al., 1996) as is the exclusive occurrence of MAO A in human thyroid (Rodriguez et al., 1990). The use of the deuterium isotope effect allows the assessment of binding specificity and provides a range of binding parameters to optimize sensitivity in organs with various combinations of blood flow, efflux and binding rates. These tracers will be useful scientific tools for the assessment of the effect of tobacco smoke exposure, drugs and other xenobiotics, genetics and other variables on MAO A and B activities directly in humans. Supported by DOE-OBER and NIH (NS-15380).

SYNTHESIS AND PRERIMINARY EVALUATION OF A C-11 LABELLED NONCOMPETITIVE ANTAGONIST FOR AMPA RECEPTOR IMAGING

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Keywords: AMPA receptor, noncompetitive antagonist, carbon-11, PET

Excessive activation of ionotropic glutamate receptors (iGluRs) may be implicated in pathophysiology of a number of neurological and psychiatric disorders. Thus, an intense effort has been focused on the developments of radioligands for imaging iGluRs by PET/SPECT. Although iGluRs are classified into three subtypes, AMPA (-amino-3-hydroxy-5-methyl-4-isoxzole propionic acid), KA (kainic acid), and NMDA (*N*-methyl-D-aspartic acid) receptors, most of the radioligands developed so far have targeted to the NMDA subtype because of the availability of selective antagonists and agonists for this receptor subtype. Recently, medicinal chemistry efforts have led to an identification of a series of quinazolin-4-ones as potent and selective noncompetitive AMPA receptor antagonists[1)], the most representative of which is CP-465,022 (Fig 1). In this paper, in order to investigate the ability of positron-emitter labeled quinazolin-4-ones to visualize AMPA receptors by PET, we have synthesized a C-11 labelled quinazolin-4-one (**1**, Fig 1) and examined for its *in vitro* and *in vivo* binding pharmacology.

The compound (**1**, IC_{50}= 31 nM) has an equal potency to CP-465,022 (IC_{50}= 36 nM) as AMPA antagonist and could be easily labelled with carbon-11. We have recently demonstrated that *in vivo* binding of a radioligand for NMDA/glycine receptors is affected by extraordinarily high levels of endogenous agonists such as glycine and D-serine. This study suggests that positron-emitter labeled noncompetitive antagonists for iGluRs might have an advantage over the corresponding competitive antagonists in *in vivo* binding because of the theoretical possibility that they are effective even at extraordinarily high level of endogenous amino acids. The quinazolin-4-one derivatives have been reported to inhibit AMPA receptors through an allosteric binding site that is distinct from the glutamate-binding site.

Radiosynthesis has been easily accomplished by the conventional methylation (30°C for 3 min) of a corresponding des-methyl precursor (**2**) with [^{11}C]methyl iodide in DMF and subsequent purification by a reversed-phase HPLC to afford a radiochemically pure (= 99%) [^{11}C]**1**. After a 5 min proton bombardment at a beam current of 15 A, 1.2 GBq of **1** was obtained in a total synthesis time of 22 min from EOB.

In vitro binding to rat brain cryosections showed a homogenous binding in the cortex, striatum, hippocampus, thalamus, and cerebellum with extremely high specific bindings (= 97%). This binding was not inhibited by endogenous and exogenous agonists, glutamate (1 mM) and AMPA (10 M), respectively. A noncompetitive AMPA/KA antagonist GYKI-52466 (IC_{50}= 22 M) did not inhibit the binding at 10 M, suggesting the different binding site of **1** from that of GYKI-52466. *In vivo* brain uptake was examined in mice and monkey. The radioligand **1** (logP= 2.60) displayed a moderate brain penetration (0.8% dose/g at 30 min post injection) in mice and showed a homogenous brain accumulation after intravenous injection in monkey.

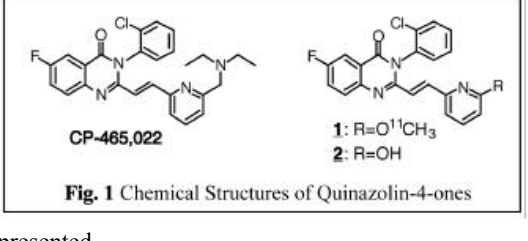

Fig. 1 Chemical Structures of Quinazolin-4-ones

The determination of *in vivo* specificity to AMPA receptors and separation of atropisomers of **1** were currently underway, and the results of which will also be presented.

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SYNTHESIS OF FLUORINE-18 SUBSTANCE P

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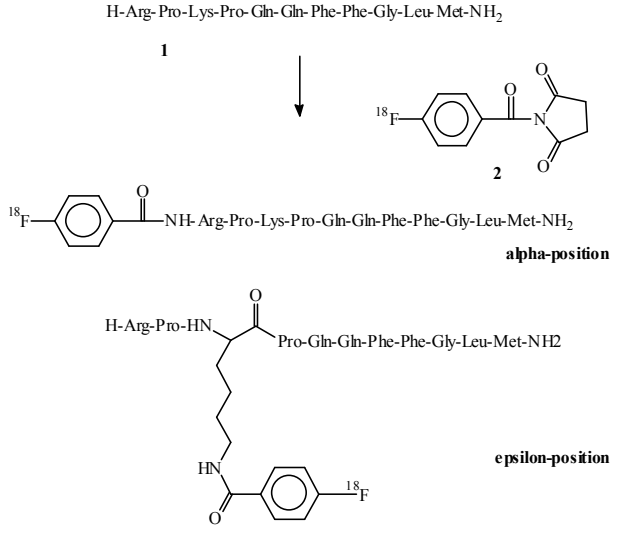
Keywords: Substance P, tachykinin, fluorine-18, peptide

The peptide substance P (SP) belongs to the family of the tachykinins and is widely distributed in the central and peripheral nervous system. Three tachykinin receptors have been identifies: NK_1, NK_2 and NK_3. SP has the greatest affinity for the NK_1 subtype. SP is considered to be a neurotransmitter or neuromodulator. Changes in SP concentration have been implicated in several diseases, such as Parkinson's disease, arhritis, inflammatory bowel disease and asthma. To develop a non-invasive diagnostic tool to measure SP receptors, SP has been radiolabelled with ^{111}In and ^{99m}Tc [1,2]. In addition, several non-peptide analogs have been prepared for PET and SPECT. Despite the anticipated low metabolic stability of SP in vivo, preliminary studies with ^{99m}Tc and ^{111}In-SP showed clear visualization of the submandibular gland. It was shown that this uptake was specific.



To prepare a radiolabelled SP for PET, the current paper describes the synthesis of the F-18 lablled peptide.

^{18}F-SP was prepared by reaction of native SP, **1** with N-succinimidyl-4-[^{18}F]fluorobenzoate, **2** (SFB). The synthetic strategy has been applied several times to other peptides or proteins.

[^{18}F]SFB was prepared as previously described [3,4] in a fully automated way with a Zymark robotic system.

[^{18}F]SFB was reacted with 0.1 mg of SP in 0.033 M borate buffer (pH 8.5) for 15 min at ambient temperature. [^{18}F]-SP labelled at the -position was prepared in a radiochemical yield of about 10%, calculated from [^{18}F]SFB. At pH=7, two radioactive isomers (at the - and the -position) of [^{18}F]-substance P were formed and were separated by RP-HPLC using gradient elution (eluent A: 0.05% TFA; eluent B: acetonitrile; A-> B in 30 min). At pH = 6, only [^{18}F]-SP labelled at the -position was formed. The identity of both regiosiomers was confirmed by mass spectrometry.

Work is in progress to evaluate both regioisomers for its potency to measure NK_1-receptors with PET.

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SYNTHESIS AND EVALUATION OF [^{18}F]-5-*tert*-BUTYL-2-[4-(3'-FLUOROPROP-1'-YNL)PHENYL-2-METHYL-1,1,-3,3-TETRAOXO-1,3-DITHIANE AS GABA$_A$ GATED CHLORIDE ION CHANNEL MARKERS

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Keywords: GABA, Dithianes, Fluorine-18, PET

Dysfunction in GABA$_A$-mediated neuromodulation has been implicated in a number of neurological abnormalities including epilepsy and anxiety disorders. Currently available *in vivo* radiotracers for the GABA$_A$ receptor are limited to [^{11}C]flumazenil and [^{123}I]iomazenil. As part of our interest in developing new radioligands which may serve as functional markers of the chloride ion channel of the GABA$_A$ receptor, we have previously evaluated numerous substituted 5-*tert*-butyl-2-phenyl-1,3-dithianes and the corresponding tetraoxodithiane derivatives, including [^{18}F]-**1**, [^{18}F]-**2**, [^{11}C]-**3** and [^{125}I]-**4**. These previous attempts yielded radiotracers with sufficient brain uptake but minimal to modest specific binding. In this communication we report on the more potent radioligand *trans*-2-[^{18}F]fluoropropynylphenyl-2-methyl-1,1,3,3-tetraoxodithiane ([^{18}F]FPMTD **6b**) as a suitable candidate for *in vivo* imaging of the GABA$_A$ receptor gated chloride ion channel.

The *cis*- and *trans*-tosylate precursors **5a** and **5b** were prepared. Radiolabelling of **5a** and **5b** with K^{18}F in the presence of Kryptofix 2.2.2 was achieved in DMF at 80^{o}C for 15 min. The crude reaction mixture was purified by HPLC (Primesphere 5C8 (4.6x30 mm), EtOH/H_2O=30/70, 2 mL/min) to provide *cis*- or *trans*-FPMTD (**6a** and **6b**) respectively in 7-35% radiochemical yield and >96% radiochemical purity.

Preliminary *in vivo* studies in mice with *trans*-isomer **6b** showed higher initial uptake into mouse brain than previously obtained for [^{18}F]-**2** and [^{11}C]-**3**, followed by a continual washout up to 120 min. At longer time points (>60 min) there was a clear heterogenous regional brain distribution with higher retention in cortex and cerebellum and lowest in pons. In contrast, the *cis*-isomer **6a**, showed equivalent initial brain uptake but rapid clearance from all regions within the first 30 min, and a homogeneous distribution at all times.

The regional brain distribution of the *trans*-isomer **6b** was consistent with the *in vivo* brain distribution of [^{11}C]flumazenil and *in vitro* binding of [^{3}H]TBOB (4-*tert*-butyl-bicycloorthobenzoate), a radioligand for the chloride ion channel of the GABA$_A$ receptor. Thus, the *trans*-isomer of [^{18}F]FPMTD appears suitable for further evaluation as a specific radioligand for the functional status of the ion channel of this important inhibitory neurotransmitter receptor.

THE PREPARATION AND BIOLOGIC EVALUATION OF I-125-7a-*O*-IA-DPN FOR POTENTIAL SPECT IMAGING OF OPIOID RECEPTORS

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Key words: Opioid receptor Diprenorphine I-125-7 -*O*-IA-DPN Distribution Rat

Objectives To synthesize I-125-7 -*O*-IA-DPN as a new opioid receptor imaging agent for SPECT study and evaluate its biological properties.

Methods 7 -*O*-stannyl-DPN was obtained from diprenorphine (DPN) by acetylating it to protect the phenolic 3-OH group of DPN and then introducing the vinylstannane into the tertiary alcohol of the 7 -side chain. [^{125}I] 7 -*O*-iodoallyl diprenorphine (7 -*O*-IA-DPN) was prepared by radio-iododestannylation under acidic condition using iodobead as an oxidant reagent, and *in vitro* and *in vivo* opioid receptor binding assays and metabolism studies were performed with Kunming mouse brains. Study of distribution in the Wistar rat's brain and naloxone inhibition was carried out. Male Wistar rats were killed by cervical dislocation at 5, 10, 20, 30 and 60 min after intravenous injection of 0.2 ml of I-125-7 -*O*-IA-DPN (74 kBq). The interesting structures in the rat brain including hippocampus, cerebellum, anterior and posterior colliculi, brain stem, striatum, frontal lobe, temporal lobe and the other parts were removed, weighed and counted. The inhibition experiment of the rats was done by injecting 20 g/0.2 ml of naloxone at 30 min before injecting I-125-7 -*O*-IA-DPN. Then the male Wistar rats were killed by cervical dislocation at 20 min post-injection, and dissected. The data were analyzed by statistical methods.

Results 7 -*O*-stannyl-DPN was proved by IR, NMR and mass spectrum analysis and I-127-7 -*O*-IA-DPN was proved by mass spectrum analysis. The radiochemical yields were 45% 48% . In TLC, Rf of 7 -*O*-IA-DPN and I-125-7 -*O*-IA-DPN was 0.83 and 0.93, respectively. In ambient temperature the radiochemical purity of I-125-7 -*O*-IA-DPN was more than 95% and 90% after 2 h and 24 h, respectively. The uptake of I-125-7 -*O*-IA-DPN in rats showed higher in anterior and posterior colliculi, striatum and hippocampus. It was low in frontal lobe, temporal lobe and brain stem and was low in cerebellum and the other parts of the brain. Among the clearance from the structures in brain, it was fastest in cerebellum. At 20 min when the uptake reached to the peak, the ratio of anterior and posterior colliculi, striatum and hippocampus to the cerebellum was 4.36, 3.7 and 3.12, respectively. There were significant differences between the inhibition experimental group using the naloxone and control.

Conclusion The designed pathway obtained with 7 -*O*-stannyl-DPN was reasonable and reliable. It was simple and rapid to iodinate 7 -*O*-stannyl-DPN using Iodobead as the oxidant reagent. The in vitro stability of I-125-7 -*O*-IA-DPN showed excellent. This novel compound has highly specific binding in anterior and posterior colliculi, striatum and hippocampus rich in opioid receptors. I-125-7 -*O*-IA-DPN appears to be a potential opioid receptor imaging agent for SPECT study.

RADIOSYNTHESIS OF [^{11}C]EMD-95885 : A HIGH AFFINITY LIGAND FOR NR2B-CONTAINING NMDA RECEPTORS

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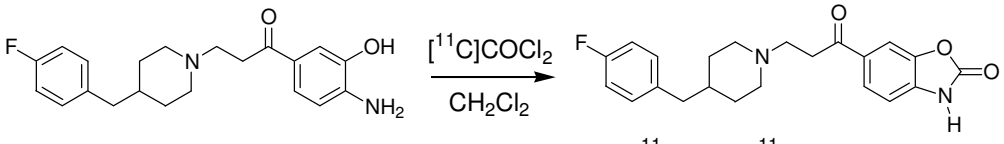
Keywords : Carbon-11, NMDA Receptor, EMD-95885

The N-Methyl-D-Aspartate (NMDA) receptor is a ligand gated ion channel composed of multiple subunits and mediates a number of physiological and pathophysiological processes in the CNS. In the brain the NR2B subunit is restricted to the forebrain regions and contains glutamate binding sites (1). EMD-95885 (**1**, namely 6-[3-[4-(fluorobenzyl)piperidino]propionyl]-[3*H*]-benzoxazol-2-one) has been reported as a high affinity ligand for the NR2B subunit (IC50 = 3.9 nM) (2). Its benzoxazolone structure permits its radiolabelling with [^{11}C]phosgene for the evaluation of its suitability as a radioligand for positron emission tomography (PET). The present work represents (a) a concise synthesis of derivative **1**, as the non radioactive reference and its precursor for labelling **2** ; (b) the labelling of EMD-95885 (**1**) with carbon-11 using [^{11}C]phosgene.

EMD-95885 (**1**) was synthesized in 2 steps from commercially available 2-benzoxazolinone. 2-Benzoxazolinone was added to a mixture of aluminium chloride in DMF followed by 3-chloropropinoyl chloride resulting in the formation of 6-(3-chloropropionyl)-[3*H*]-benzoxazol-2-one. This product was then treated with 4-(4-fluorobenzyl)piperidine in EtOH containing NEt_3 to yield the benzoxazolone **1** in 65% yield. Benzoxazolone ring-opening was performed using tert-butyldicarbonate (in THF containing NEt_3 and DMAP) followed by acid hydrolysis (TFA, dichloromethane) and cleanly give **2** as the precursor for labelling.



2 [^{11}C]-**1** [^{11}C]EMD-99885

[^{11}C]Phosgene was synthesized from [^{11}C]methane via [^{11}C]carbon tetrachloride using a combination of published processes (3,4). The cyclization reaction of [^{11}C]phosgene and the aminophenol derivative **2** in dichloromethane at room temperature was rapid and afforded pure [^{11}C]-**1** after HPLC purification (column: Hewlett Packard Zorbax RX-Sil, 250 × 9.4 mm; 5 µm; eluent: CH_2Cl_2/MeOH/NH_3 93/7/0.5). The total synthesis time was 28-30 minutes and the specific radioactivity was about 1 Ci/µmol (37 GBq/µmol) at the end of the synthesis.

Pharmacological profile of [^{11}C]-**1** is currently being evaluated *in vivo*. Biodistribution studies and brain radioactivity monitoring using intracerebral radiosensitive β-microprobes in rodents are underway.

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TRACER FOR ANGIOGENESIS IMAGING: POTENTIAL TARGETS AND RECENT PROGRESS

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Angiogenesis, the formation of new blood vessels is an absolute requirement for the growth of malignant tumors. Thus, antiangiogenic agents inhibiting this process provide new approaches to treating tumors. Most of them target growth factor receptors, matrix metalloproteinases and integrins or use endogenous inhibitors (e.g. Endostatin). However, clinical trials based on these concepts appears to be effective only in a limited number of patients. Moreover, because antiangiogenic therapies are primarily cytostatic and not cytotoxic, established methods for the determination of tumor response to chemo- and radiotherapy are not appropriate for monitoring these therapeutic approaches. New strategies are focused on the development of radiolabeled specific angiogenic markers, which should provide helpful information for drug development and therapy control. Based on the therapeutic approaches molecular targets for such tracers include vascular endothelial growth factor receptors, tyrosine kinases, cryptic angiogensis inhibitors, matrix metalloproteinases and integrins.

The ED-B+ fibroncetin isoform is an extracellular matrix protein which is important in vascular proliferation and is widely expressed on neoplastic tissue but shows a highly restricted distribution in normal adult tissue. It has been demonstrated that a radioiodinated single chain fragment, binding selectively to the ED-B+ domain, revealed high and selective accumulation in tumor vessels in different animal/tumor models.

Using phage display libraries a disulfide bridged decapeptide which selectively inhibits the matrix metalloproteinases MMP-2 and MMP-9 involved in matrix degradation during migration of the endothelial cells was found. Moreover, CTTHWGFTLC suppresses migration of tumor and endothelial cells in vitro. In addition, presented on phages the peptide allows homing of the phages in the tumor vasculature. Based on this promising results a undecapeptide including a D-Tyr at the C-terminal end and a DOTA-conjugated peptide were synthesized. In vitro assays showed for the modified peptides same inhibitory capacities as found for corresponding reference compounds. However, further studies with the first tracer demonstrated low metabolic stability and high lipophilicity resulting in high activity concentration in liver and kidneys in vivo. For the later tracer, preliminary microPET studies with tumor bearing nude mice showed significant activity accumulation in the tumor. Based on MMP inhibitors also non-peptidic tracer have been developed. In vitro data are promising, however, further evaluation in vivo have to be carried out to demonstrate the potential of this tracer class to monitor angiogenesis.

At the moment, most effort is being concentrated on developing radiolabeled $\alpha v\beta 3$ integrin antagonists. The $\alpha v\beta 3$ integrin mediates migration of activated endothelial cells through the basement membrane during formation of new blood vessels. It is highly expressed on activated endothelial cells but not on quiescent endothelial cells of established vessels. A variety of $\alpha v\beta 3$ antagonists have been developed. First the iodinated derivative [^{125}I]cyclo(-Arg-Gly-Asp-D-Tyr-Val-), which showed high $\alpha v\beta 3$ affinity and selectivity in vitro and receptor specific tumor accumulation in vivo, was synthesized. The unfavorable pharmacokinetic properties have been improved by introduction of sugar moieties. The resulting glycopeptides [*I]Gluco-RGD and [^{18}F]Galacto-RGD showed a clearly reduced activity concentration in the liver and an increased activity accumulation in the tumor. Small animal PET studies using different murine tumor models demonstrated that [^{18}F]Galacto-RGD allows non-invasive determination of the $\alpha v\beta 3$ expression on receptor positive tumors as well as in tumor vasculature in models were the tumor itself do not express $\alpha v\beta 3$. These studies indicate that non-invasive imaging of tumor-induced angiogenesis may be possible. This is confirmed by recent experiments with the Rip-Tag model, a transgenic mouse