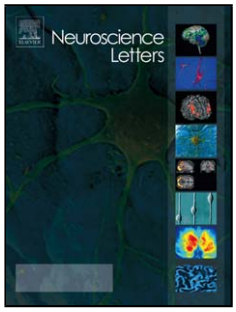
Accepted Manuscript



Title: The tricyclic antidepressant desipramine inhibits T_3 import into primary neurons

Authors: Stephan Roth, Anita Kinne, Ulrich Schweizer

PII: S0304-3940(10)00516-1
DOI: doi:10.1016/j.neulet.2010.04.055
Reference: NSL 27019

To appear in: *Neuroscience Letters*

Received date: 10-2-2010
Revised date: 6-4-2010
Accepted date: 22-4-2010

Please cite this article as: S. Roth, A. Kinne, U. Schweizer, The tricyclic antidepressant desipramine inhibits T_3 import into primary neurons, *Neuroscience Letters* (2008), doi:10.1016/j.neulet.2010.04.055

The tricyclic antidepressant desipramine inhibits T_3 import into primary neurons

Stephan Roth*, Anita Kinne*, Ulrich Schweizer#

Institute for Experimental Endocrinology, Charité-Universitätsmedizin Berlin, 13353 Berlin, Germany

Text-Pages: 9

Nr. of Figures: 3

Nr. of Tables: 0

*these two authors have contributed equally to the experiments presented in this manuscript

#Corresponding author: Institut für Experimentelle Endokrinologie, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany.

Tel.: +49 30 450524080; Fax. +49 30 450524922

E-mail address: ulrich.schweizer@charite.de

Abstract

Transport of thyroid hormones across the plasma membrane is required for binding to their nuclear receptors. Monocarboxylate transporter 8 (MCT8) is a plasma membrane thyroid hormone transport protein, which has recently gained much attention, since mutations in *MCT8* are associated with severe mental retardation in patients afflicted with the Allan-Herndon-Dudley syndrome. MCT8 is expressed along the blood-brain-barrier and on central neurons. We have found that desipramine (DMI), a tricyclic antidepressant, acts as an inhibitor of thyroid hormone transport by MCT8. Uptake of 3,5,3'-triiodo-L-thyronine (T_3) into primary cortical neurons could be blocked with desipramine as well as with the known, but unspecific, inhibitor bromosulphtalein (BSP). T_3 uptake by neurons derived from *Mct8*-deficient cells was not further decreased by DMI. In a heterologous expression system, both human MCT8 and its close homolog, MCT10, were sensitive to inhibition by DMI. Kinetic experiments demonstrated a non-competitive mode of inhibition. Numerous interactions between thyroid hormones, depressive symptoms, and antidepressant treatments have been reported in the literature. Our findings add to the evidence that antidepressant drugs may affect CNS thyroid hormone function.

Keywords: Tricyclic antidepressants, Desipramine, T_3-Transport, Tofranil

Thyroid hormones are important regulators of brain development and function. Key processes like neurogenesis, neuronal migration, formation of dendritic spines, and myelination are thyroid hormone-dependent [3, 15, 37]. The main product of the thyroid, thyroxine (3,5,3',5'-tetraiodo-L-thyronine, T_4) is a prohormone. Only its derivative 3,5,3'-triiodo-L-thyronine (T_3) is capable of high affinity binding and activating nuclear T_3-receptors. Local activation of T_4 to T_3 and inactivation of both hormones is mediated by deiodinases, three selenium-dependent isoenzymes [28]. In the brain, type III 5-deiodinase (Dio3) catalyzes thyroid hormone-inactivating reactions, while activation of T_4 to T_3 is mediated by type II 5'-deiodinase (Dio2). Homeostatic regulation of deiodinases helps maintain appropriate cellular T_3 levels during brain development and function. Only recently, it has been widely accepted that thyroid hormones, owing to their chemical nature as zwitter-ionic amino acid derivatives, require plasma membrane transport proteins. One of these, monocarboxylate transporter 8 (MCT8), is mutated in Allan-Herndon-Dudley syndrome, a severe psychomotor retardation [12, 13]. We have shown that T_3-uptake into cortical neurons derived from *Mct8*-deficient mice is severely diminished [35].

Thyroid hormones also influence mood. Severe hypothyroidism may mimic all kinds of symptoms of major depressive disorder [17]. In major depression, T_3- and T_4-augmentation were used to improve the response of clinical non-responders to antidepressant therapy and to accelerate tricyclic antidepressant response [22, 27]. Another enigmatic aspect is the apparent tolerance of depressed patients towards high dose thyroxin administration which points to a profoundly altered thyroid hormone response in these patients [2, 30]. Most of these studies used the tricyclic antidepressant drug imipramine or its active metabolite, desipramine (DMI), which were introduced more than fifty years ago [4, 31, 33]. Despite extensive research efforts and several interesting working hypotheses, the biochemical mechanisms underlying the effects of antidepressant therapies are still unknown [8, 26]. In basic research studies it was found that DMI stimulates Dio2 activity in rat brain [7]. Later, it was shown that this

induction of Dio2 activity in the cortex was specific for DMI and did not occur after treatment with fluoxetine, haloperidol, and tranylcypromine [10]. Since in this study, cortical T_4 content was dose-dependently diminished by DMI, we hypothesized that this result may be explained by a blockade by DMI of thyroid hormone transport, either into Dio2-expressing astrocytes or across the blood-brain-barrier. In either case, reduced thyroid hormone levels would lead to an induction of Dio2 activity. MCT8 is not only located in neurons, but also along elements of the blood-brain- and blood-cerebrospinal fluid-barrier [25, 34, 35] and astrocytes (U.S., *unpublished data*). In order to test whether DMI acts as an inhibitor of cellular T_3 uptake, we assessed ^{125}I-T_3 uptake into primary neurons in the presence or not of DMI along with bromosulphtalein (BSP), an inhibitor of MCT8 [35] and other transporters, e.g. Lat1 [11]. In short, primary cortical neurons dissected from embryonic day 15 mouse brains were cultured at $1.5x10^5$ per cm^2 for two weeks and incubated in the presence or not of 1 mM BSP or DMI for 3 minutes with 10 nM ^{125}I-T_3. Both substances reduced T_3-uptake into neurons to more than 50% (Fig. 1). Incubation with BSP or DMI did not significantly reduce T_3 uptake into *Mct8*-deficient neurons. Therefore, we concluded that both compounds acted primarily by inhibition of neuronal MCT8.

In order to test whether human MCT8 is similarly sensitive to inhibition by DMI, we took advantage of a heterologous expression system. Madin-Darby canine kidney (MDCK-1) cells exhibit only little endogenous T_3 uptake. Therefore, MDCK-1 cells were stably transfected with a cDNA encoding N-terminally HA-tagged hMCT8 [35]. Because MCT10 is so closely related to MCT8 and was recently shown to mediate import and export of thyroid hormones [14], we also established MDCK-1 cells stably expressing HA-hMCT10 (Fig. 2A,C, insets). We then incubated MCT8- or MCT10-expressing cell lines with DMI and measured ^{125}I-T_3 uptake. BSP is a competitive inhibitor and served as positive control. Background T_3 uptake by transfected MDCK-1 cells with empty vector was subtracted. As expected, both MCTs were inhibited by DMI (Fig. 2A,C). We then determined K_i values for DMI using MCT8- and

MCT10- expressing cells. In standard Dulbecco's Modified Eagle Medium (DMEM; without addition of serum during T_3 incubation), K_i for DMI on MCT8 was 273 µM and could not be calculated for MCT10 (Fig. 2B, D). We reasoned that amino acids present in DMEM interfered with T_3 uptake by MCT10, since MCT10 is also known as TAT1, the T-type amino acid transporter specific for aromatic amino acids [14]. Therefore, we determined K_i again, but in a physiological buffer lacking amino acids. The means of two experiments resulted in a K_i for DMI of 495 µM on MCT8 and 390 µM on MCT10 (Fig. 2B, D). Interestingly, our data point to potential competition of a medium constituent with T_3 at MCT8. Aromatic amino acids, however, can be excluded and so far, only iodothyronines were identified as MCT8 substrates [12].

We then investigated the mode of inhibition by DMI for MCT8. For this purpose, we determined the K_m for T_3 on MCT8 in the presence of different concentrations of DMI. These experiments were performed in physiological buffer at DMI concentrations of 390 µM (K_i) and 130 µM ($K_i/3$). After plotting the data into an Eadie-Hofstee diagram, the curves were essentially parallel (K_m=1.2 µM without DMI; K_m =1.02 µM at 130 µM DMI and K_m =1.37 µM at 390 µM DMI). Therefore, we conclude that inhibition of MCT8-mediated T_3 uptake follows a non-competitive mechanism (Fig. 3).

In aggregate, the results presented here support the idea that DMI is an inhibitor of MCT8-mediated thyroid hormone transport. MCT8 is expressed in several cell types and organs, not only in neurons and hypothalamic tanycytes [18, 35]. Roberts and coworkers have demonstrated MCT8 expression along the blood-brain-barrier [25]. Accordingly, it was shown that T_3 import into the brain is highly dependent on Mct8 in the mouse [34]. High sex-hormone-binding-globulin and low plasma cholesterol in *MCT8*-mutant patients and *Mct8*-deficient mice support that T_3-import into the liver does not depend on MCT8 [9, 34]. In contrast, some tissues like pituitary and heart are apparently resistant to thyroid hormone in *MCT8*-deficiency, since plasma TSH is inappropriately high in face of high circulating T_3

levels and tachycardia is not severe. Together, these data support the view that cellular thyroid hormone status is modulated by the expression of thyroid hormone transporter proteins. Such heterogeneity regarding the expression of thyroid hormone transport proteins may also exist in subdivisions of the brain, but has not been systematically studied. Previous studies showed a strong stimulating effect of DMI on Dio2 activity in different rat brains [7]. In agreement with this finding, T_4 tissue concentrations were found decreased, but – surprisingly – T_3 concentrations remained constant (except in the amygdala) [23]. Although our data are not sufficient to prove that the mode of action of DMI as an antidepressant drug involves exclusively the local modulation of thyroid hormone transport and metabolism, our data suggest that such an effect should be considered and addressed in future experiments.

Many studies have addressed whether the antidepressant effect of DMI is linked to changes in thyroid hormone metabolism or not [1, 6, 19]. Some data suggested that the thyroid axis of DMI responders and non-responders reacts differently, i.e. serum T_4 levels decreased upon DMI treatment in responders, but not non-responders [29]. A meta-analysis on studies investigating the effect of co-administration of T_3 and DMI supported a positive effect on therapeutic success. Five of six studies analyzed found a statistically significant effect for reduced latency until a response to DMI could be noticed [1]. Moreover, T_3 treatment turned some of the DMI non-responders into responders while showing no adverse effects [20, 21]. The effects on T_3 transport of DMI occurred at rather high concentrations *in vitro*. Therapeutic serum levels of DMI in humans are in the nM range. The DMI doses applied in animal studies [10, 23] were tenfold to twentyfold higher than prescriptions in humans. Moreover, we do not know whether DMI reaches higher local concentrations in the brain interstitium. While DMI inhibits serotonin uptake at sub-micromolar concentrations, dopamine uptake is still incompletely inhibited at 30 µM [36], not far from inhibition of T_3 uptake. In addition, the non-competitive mode of inhibition of MCT8 by DMI may lead to gradually increasing inhibition with time of exposure to drug.

There is some evidence that thyroid hormones may be involved in the as yet unknown mechanism of action of antidepressant treatments. We, report here a novel effect of an antidepressant drug, inhibition of MCT8-mediated thyroid hormone transport. DMI was shown to inhibit T_3 import into brain [16]. Other reports demonstrated DMI inhibition of L-type amino acid transporters [24, 32]. Since MCT8 and L-type amino acid transporters are expressed in neurons [18, 25], astrocytes [5], and along the blood-brain-barrier [35], DMI may act on multiple targets at several cell types. To date, no specific MCT8 inhibitor is known. Therefore, research on thyroid hormone transport may benefit from the identification of specific pharmacological inhibitors for MCT8 and other thyroid hormone transporters. Future experiments, should address whether DMI acts as well on other thyroid hormone transporters e.g. of the OATP family.

Acknowledgements

Financial support was provided by Deutsche Forschungsgemeinschaft DFG, project SFB665 and Charité-Universitätsmedizin Berlin. We greatfully acknowledge critical reading of the manuscript by Prof. Dr. Andreas Baumgartner, Charité-Universitätsmedizin Berlin. We also thank two anonymous reviewers for their helpful comments.

Figure-Legends

Fig 1: Chemical structure of 3,5,3'-triiodo-L-thyronine (T_3), bromosulphtalein (BSP), and desipramine (DMI) (A). Percental T_3 uptake inhibition on cultured primary neurons with 1 mM BSP or 1 mM DMI at 10 nM ^{125}I-T_3 concentration in neuronal media. T_3 uptake without inhibitor was set at 100%. Incubation was performed for 3 min in triplicate cell culture wells. *** $p<0.001$. One-way ANOVA followed by Bonferronis's post-test (B).

Fig 2: Specific inhibitory characteristics of desipramine on MCT8 and MCT10 in stably transfected MDCK-1 cells. ^{125}I-T_3 uptake under treatment of 1 mM desipramine (DMI) or 1 mM bromosulphtalein (BSP). Incubation time was 3 min for MCT8 and 5 min for MCT10. Radioactivity of cells transfected with empty vector was set at zero, while ^{125}I-T_3 uptake lacking inhibitors was set at 100%. Data represent +/- SEM of T_3 uptakes performed in triplicate cell culture wells. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Two-way ANOVA followed by Bonferroni's post-test (A, C). Expression of HA-tagged MCT8 and MCT10 was determined by immunoblotting (- empty vector, + HA-MCT8/MCT10) (A, C, insets). Determination of K_i values for DMI on MCT8 or MCT10 in Dulbecco's Modified Eagle Medium (DMEM) and physiological buffer (B, D). Data reported is representative of one out of two independent experiments with similar results.

Fig 3: Mode of inhibition by desipramine for MCT8 by Eadie-Hofstee transformed data. Stably transfected MDCK-1 cells were incubated with ranging from 500 nM to 12 µM in physiological buffer under different desipramine concentrations (390 µM=K_i, 130 µM=K_i/3, and w/o desipramine). Data represents cellular ^{125}I-T_3 uptake +/- SEM performed in triplicate cell culture wells.

References

1 Altshuler, L.L., Bauer, M., Frye, M.A., Gitlin, M.J., Mintz, J., Szuba, M.P., Leight, K.L. and Whybrow, P.C., Does thyroid supplementation accelerate tricyclic antidepressant response? A review and meta-analysis of the literature, American Journal of Psychiatry, 158 (2001) 1617-22.

2 Bauer, M., Hellweg, R., Graf, K.J. and Baumgartner, A., Treatment of refractory depression with high-dose thyroxine, Neuropsychopharmacology, 18 (1998) 444-55.

3 Bernal, J. and Nunez, J., Thyroid hormones and brain development, European Journal of Endocrinology, 133 (1995) 390-8.

4 Blier, P. and de Montigny, C., Current advances and trends in the treatment of depression, Trends in Pharmacological Sciences, 15 (1994) 220-6.

5 Blondeau, J.P., Beslin, A., Chantoux, F. and Francon, J., Triiodothyronine is a high-affinity inhibitor of amino acid transport system L1 in cultured astrocytes, Journal of Neurochemistry, 60 (1993) 1407-13.

6 Brady, K.T. and Anton, R.F., The thyroid axis and desipramine treatment in depression, Biological Psychiatry, 25 (1989) 703-9.

7 Campos-Barros, A., Meinhold, H., Stula, M., Muller, F., Kohler, R., Eravci, M., Putzien, O. and Baumgartner, A., The influence of desipramine on thyroid hormone metabolism in rat brain, Journal of Pharmacology and Experimental Therapeutics, 268 (1994) 1143-52.

8 Cowen, P.J., Serotonin and depression: pathophysiological mechanism or marketing myth?, Trends in Pharmacological Sciences, 29 (2008) 433-6.

9 Dumitrescu, A.M., Liao, X.H., Weiss, R.E., Millen, K. and Refetoff, S., Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct) 8-deficient mice, Endocrinology, 147 (2006) 4036-43.

10 Eravci, M., Pinna, G., Meinhold, H. and Baumgartner, A., Effects of pharmacological and nonpharmacological treatments on thyroid hormone metabolism and concentrations in rat brain, Endocrinology, 141 (2000) 1027-40.

11 Friesema, E.C., Docter, R., Moerings, E.P., Verrey, F., Krenning, E.P., Hennemann, G. and Visser, T.J., Thyroid hormone transport by the heterodimeric human system L amino acid transporter, Endocrinology, 142 (2001) 4339-48.

12 Friesema, E.C., Ganguly, S., Abdalla, A., Manning Fox, J.E., Halestrap, A.P. and Visser, T.J., Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter, Journal of Biological Chemistry, 278 (2003) 40128-35.

13 Friesema, E.C., Grüters, A., Biebermann, H., Krude, H., von Moers, A., Reeser, M., Barrett, T.G., Mancilla, E.E., Svensson, J., Kester, M.H., Kuiper, G.G., Balkassmi, S., Uitterlinden, A.G., Köhrle, J., Rodien, P., Halestrap, A.P. and Visser, T.J., Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation, Lancet, 364 (2004) 1435-7.

14 Friesema, E.C., Jansen, J., Jachtenberg, J.W., Visser, W.E., Kester, M.H. and Visser, T.J., Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10, Molecular Endocrinology, 22 (2008) 1357-69.

15 Gomes, F.C., Maia, C.G., de Menezes, J.R. and Neto, V.M., Cerebellar astrocytes treated by thyroid hormone modulate neuronal proliferation, Glia, 25 (1999) 247-55.

16 Gordon, J.T., Tomlinson, E.E., Greenberg, J. and Dratman, M.B., An acute dose of desmethylimipramine inhibits brain uptake of [125I]3,3',5-triiodothyronine (T3) in thyroxine-induced but not T3-induced hyperthyroid rats: implications for tricyclic antidepressant therapy, Journal of Pharmacology and Experimental Therapeutics, 270 (1994) 111-7.

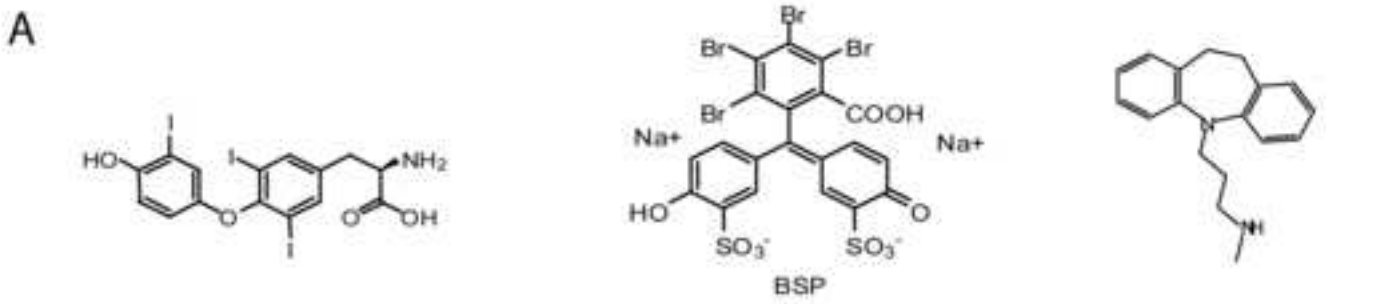
17 Hall, R.C., Psychiatric effects of thyroid hormone disturbance, Psychosomatics, 24 (1983) 7-11, 15-8.
18 Heuer, H., Maier, M.K., Iden, S., Mittag, J., Friesema, E.C.H., Visser, T.J. and Bauer, K., The Monocarboxylate Transporter 8 Linked to Human Psychomotor Retardation Is Highly Expressed in Thyroid Hormone-Sensitive Neuron Populations, Endocrinology, 146 (2005) 1701-1706.
19 Joffe, R.T. and Singer, W., Antidepressants and thyroid hormone levels, Acta Medica Austriaca, 19 Suppl 1 (1992) 96-7.
20 Joffe, R.T. and Singer, W., A comparison of triiodothyronine and thyroxine in the potentiation of tricyclic antidepressants, Psychiatry Research, 32 (1990) 241-51.
21 Joffe, R.T., Singer, W., Levitt, A.J. and MacDonald, C., A placebo-controlled comparison of lithium and triiodothyronine augmentation of tricyclic antidepressants in unipolar refractory depression, Archives of General Psychiatry, 50 (1993) 387-93.
22 Nemeroff, C.B., Augmentation regimens for depression, Journal of Clinical Psychiatry, 52 Suppl (1991) 21-7.
23 Pinna, G., Broedel, O., Eravci, M., Stoltenburg-Didinger, G., Plueckhan, H., Fuxius, S., Meinhold, H. and Baumgartner, A., Thyroid hormones in the rat amygdala as common targets for antidepressant drugs, mood stabilizers, and sleep deprivation, Biological Psychiatry, 54 (2003) 1049-59.
24 Ritchie, J.W., Collingwood, C.J. and Taylor, P.M., Effect of hypothyroidism on pathways for iodothyronine and tryptophan uptake into rat adipocytes, American Journal of Physiology - Endocrinology and Metabolism, 280 (2001) E254-9.
25 Roberts, L.M., Woodford, K., Zhou, M., Black, D.S., Haggerty, J.E., Tate, E.H., Grindstaff, K.K., Mengesha, W., Raman, C. and Zerangue, N., Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier, Endocrinology, 149 (2008) 6251-61.
26 Sanacora, G., New understanding of mechanisms of action of bipolar medications, Journal of Clinical Psychiatry, 69 Suppl 5 (2008) 22-7.
27 Schwarcz, G., Halaris, A., Baxter, L., Escobar, J., Thompson, M. and Young, M., Normal thyroid function in desipramine nonresponders converted to responders by the addition of L-triiodothyronine, American Journal of Psychiatry, 141 (1984) 1614-6.
28 Schweizer, U., Weitzel, J.M. and Schomburg, L., Think globally: act locally. New insights into the local regulation of thyroid hormone availability challenge long accepted dogmas, Molecular and Cellular Endocrinology, 289 (2008) 1-9.
29 Sokolov, S.T., Kutcher, S.P. and Joffe, R.T., Changes in thyroid hormone levels associated with desipramine response in adolescent depression, Progress in Neuro-Psychopharmacology & Biological Psychiatry, 20 (1996) 1053-63.
30 Stancer, H.C. and Persad, E., Treatment of intractable rapid-cycling manic-depressive disorder with levothyroxine. Clinical observations, Archives of General Psychiatry, 39 (1982) 311-2.
31 Stip, E., Happy birthday neuroleptics! 50 years later: la folie du doute, European Psychiatry, 17 (2002) 115-9.
32 Taylor, P.M. and Ritchie, J.W., Tissue uptake of thyroid hormone by amino acid transporters, Best Practice & Research Clinical Endocrinology & Metabolism, 21 (2007) 237-51.
33 Thornley, B. and Adams, C., Content and quality of 2000 controlled trials in schizophrenia over 50 years, British Medical Journal, 317 (1998) 1181-4.
34 Trajkovic, M., Visser, T.J., Mittag, J., Horn, S., Lukas, J., Darras, V.M., Raivich, G., Bauer, K. and Heuer, H., Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8, Journal of Clinical Investigation, 117 (2007) 627-35.

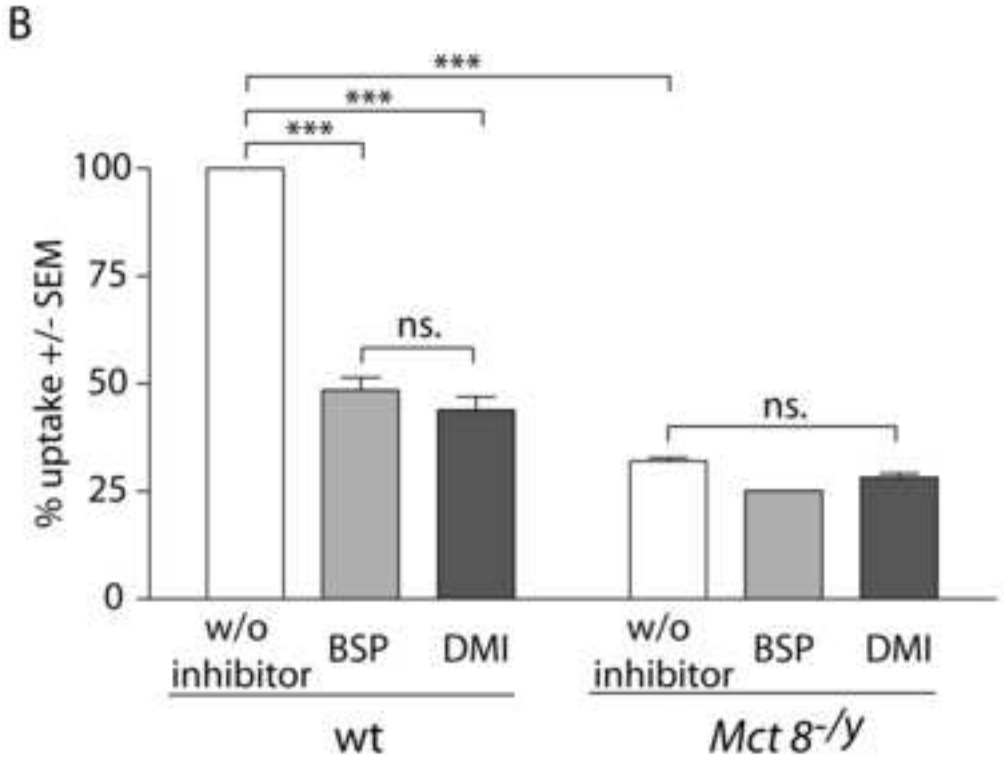
35 Wirth, E.K., Roth, S., Blechschmidt, C., Holter, S.M., Becker, L., Racz, I., Zimmer, A., Klopstock, T., Gailus-Durner, V., Fuchs, H., Wurst, W., Naumann, T., Brauer, A., de Angelis, M.H., Köhrle, J., Grüters, A. and Schweizer, U., Neuronal 3',3,5-triiodothyronine (T3) uptake and behavioral phenotype of mice deficient in Mct8, the neuronal T3 transporter mutated in Allan-Herndon-Dudley syndrome, Journal of Neuroscience, 29 (2009) 9439-49.

36 Zhou, Z., Zhen, J., Karpowich, N.K., Goetz, R.M., Law, C.J., Reith, M.E. and Wang, D.N., LeuT-desipramine structure reveals how antidepressants block neurotransmitter reuptake, Science, 317 (2007) 1390-3.

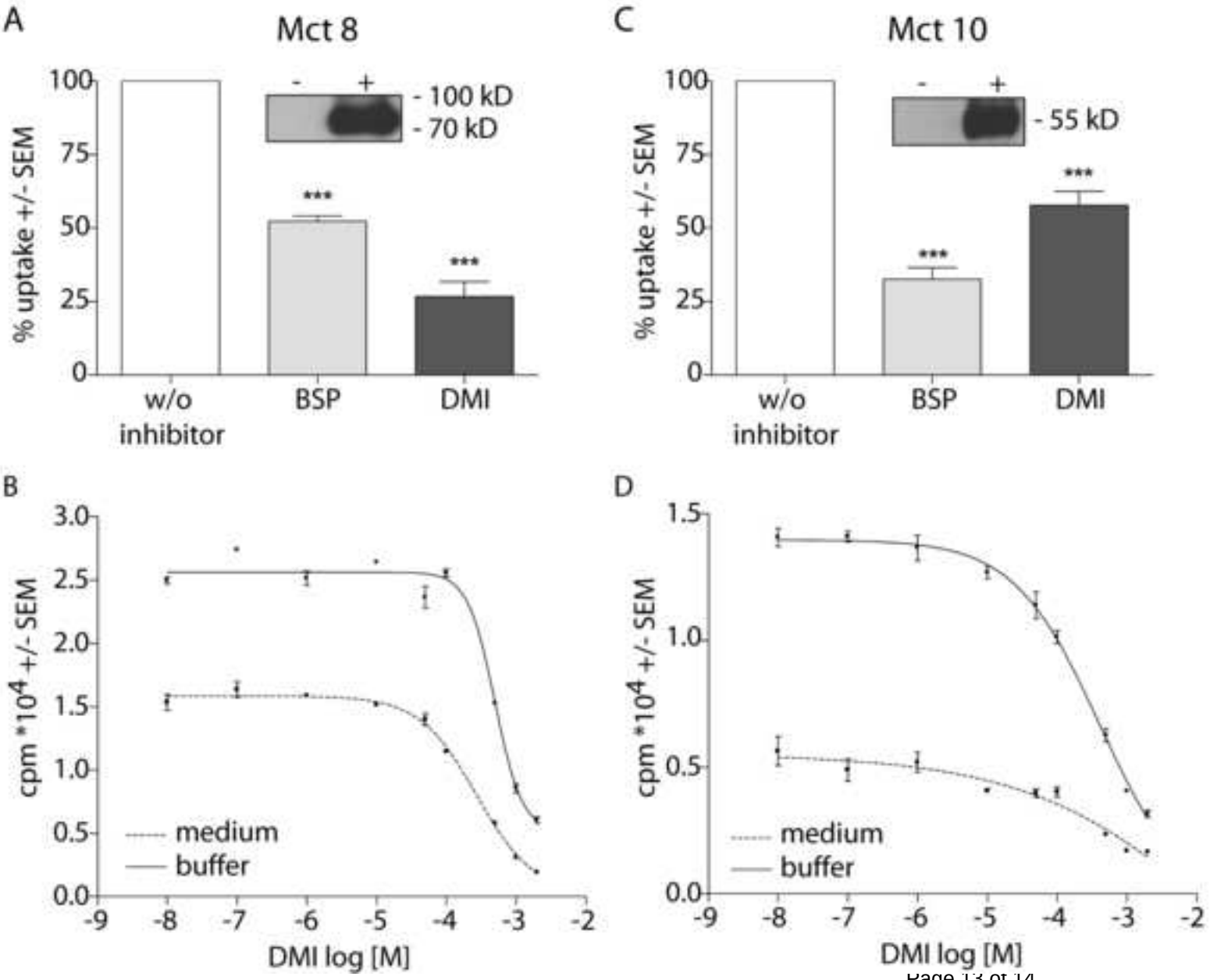
37 Zoeller, R.T. and Rovet, J., Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings, Journal of Neuroendocrinology, 16 (2004) 809-18.

Roth et al. Fig. 1





Roth et al. Fig. 2



Roth et al. Fig. 3