Effect of Nicotine on Type 2 Deiodinase Activity in Cultured Rat Glial Cells

ATSUSHI GONDOU, NAGAOKI TOYODA, MITSUSHIGE NISHIKAWA, TOSHINAGA YONEMOTO, NORIKO SAKAGUCHI, TOSHIKO TOKORO, AND MITSUO INADA

Second Department of Internal Medicine, Kansai Medical University, Osaka 570-8507, Japan

Abstract. Intracellular generation of triiodothyronine (T3) from thyroxine (T4) by type 2 deiodinase (D2) in the mammalian brain, plays a key role in thyroid hormone action. The presence of D2 in rat astrocytes suggests the importance of glial cells in the regulation of intracellular T3 levels in the rat central nervous system (CNS). To analyze further the factors that regulate D2 activity in the CNS, we investigated the effects of nicotine and of mecamylamine, which inhibits the binding of nicotine with nicotinic acetylcholine receptors, on D2 activity in cultured mixed glial cells of the rat brain. We incubated cultured mixed glial cells obtained from neonatal Wistar rats in the presence of 10 mM dithiothreitol, 2 nM [125I] reverse T3 and 1 mM 6-N-propyl-2-thiouracil for 2 h at 37 °C, and the released 125I− was counted in a γcounter. D2 activity of cultured cells was dependent on the temperature and the amount of protein. The basal D2 activity of rat mixed glial cells was 1.9 ± 0.2 fmol of I− released/mg protein/h (mean ± SEM). The addition of 10−11, 2 x 10−11, 10−10, and 10 M nicotine significantly increased D2 activity to approximately 2.2-, 2.4, 3.5- and 2.9-fold the basal level, respectively. D2 activity stimulated by 10−8 M nicotine (2.5-fold) reached a peak after 9 h incubation. The stimulatory effect of nicotine was completely blocked by 10−6 M mecamylamine. In conclusion, nicotine increases D2 activity probably via nicotinic acetylcholine receptors, and may influence brain function, at least in part, by affecting thyroid hormone metabolism.

Key words: Deiodinase, Nicotine, Mecamylamine, Glial cells, Brain

THYROID hormones play an important role in brain maturation, and the deficiency of these hormones during development leads to irreversible brain damage [1]. As hypothyroidism sometimes impairs mental function, if the thyroid hormone level in the brain remains in the optimal range it will contribute to the maintenance of fair brain function. Outer-ring deiodination of thyroxine (T4) to triiodothyronine (T3) is essential for thyroid hormone activation, and this process is catalyzed by iodothyronine 5'-deiodinase. In the central nervous system (CNS), two outer-ring deiodinases have been identified [2–5], type 1 and type 2, sensitive and insensitive, respectively, to 6-N-propyl-2-thiouracil (PTU). Type 1 deiodinase is distributed in various organs including the liver and kidneys, and plays a role in maintaining circulating T3 levels. On the other hand, type 2 deiodinase (D2) is responsible for the generation of intracellular T3 and has been reported as playing a central role in the activity of the thyroid hormone in the rat brain [2, 6, 7]. The distribution of D2 is limited to some regions of the brain, such as the pituitary and pineal glands, and brown adipose tissue in rats [8, 9]. The discovery of D2 activity in rat astrocytes suggests its importance in the regulation of intracellular T3 levels in glial cells.
Healthy glial cells are necessary for the maintenance of neuronal function, and therefore D2 could also have an important influence on neuronal function.

It was reported that catecholamines, which act as neurotransmitters, increase D2 activity via the cyclic adenosine 3′, 5′-monophosphate (cAMP) pathway [10]. But it is not reported whether acetylcholine (another neurotransmitter) influences D2 activity. On the other hand, Cornelia et al. [11] reported that nicotine intake through smoking was significantly less common in patients with dementia, and Shimohama et al. [12] reported a decrease in nicotinic receptor in Alzheimer-type dementia, so that thyroid hormone action via nicotinic receptor may affect on the mental function.

In this paper, we focused on the effect of nicotine, and analyzed the effect of nicotine and mecamylamine, which inhibits the binding of nicotine to nicotinic acetylcholine receptors, on D2 activity in rat glial cells.

Materials and Methods

The materials used were purchased from the following sources: 125I-labeled reverse T3 ([125I]rT3) with a specific activity of ~37 KBq/µl from Amersham (Arlington Heights, IL); rT3 from Calbiochem (La Jolla, CA); DL-dithiothreitol (DTT), PTU, nicotine, and mecamylamine from Sigma (St. Louis, MO); fetal bovine serum (FBS) from Flow (McLean, UA); Dowex 50W × 2 from Muromachi Chemical (Tokyo, Japan); Sephadex LH 20 from Pharmacia (Uppsala, Sweden); all other reagents were of the highest purity and were commercially available.

[125I]rT3 was purified by passing it through small LH-20 columns before use and contained less than 1% iodine as the only contaminant.

Cell culture

The whole brain of neonatal Wistar rats (1–2 days old) was placed in calcium-free Hanks’ solution (pH 7.4). After removing the meninges and blood vessels, the whole brain was minced, filtered through a nylon mesh (250 and 120 µm) and centrifuged at 725 × g for 5 min. The cell pellet was resuspended in 15 ml of 15% FBS in Dulbecco’s modified Eagle’s medium (DMEM), and the mixed glial cell suspension (2–4 × 10^5/ml) was incubated at 37 °C under water-saturated 5% CO₂ and 95% air. The culture medium was changed every three days. Neurons, which did not adhere to the bottom of the culture dish, were removed. The cell monolayers prepared thus were composed of flat polygonal cells with a few clusters of branched cells. After 9 days of incubation in DMEM plus 15% FBS, the mixed glial cells were used for the assays.

D2 activity assay

D2 activity was assayed as described previously with minor modifications [13]. Briefly, at the end of the incubation, the medium was removed, the cells were washed twice with PBS, 600 µl/well of ice-cold 10 mM DTT-buffer (0.1 M potassium phosphate containing 1 mM EDTA, pH 7.4) was added, and the cells were sonicated for 30 sec. Then 20 µl of buffer containing 2 nM [125I]rT3 and 1 mM PTU (final concentration) was added to 80 µl of the homogenate, which was then incubated for 2 h at 37 °C. At the end of the incubation, 2% bovine serum albumin and 10% trichloracetic acid were added. The homogenate was centrifuged, and after the radiiodine released was separated on a Dowex 50W × 2 column equilibrated with 10% acetic acid, the separated 125I⁻ was counted in a γ-counter. The protein content was measured by the method of Bradford [14], and D2 activity was expressed as fmol of I⁻ released/mg protein/h. Data were presented as the mean ± SEM, and statistical evaluation was performed by the Student’s t-test.

Results

Effect of nicotine on D2 activity

In the preliminary study, [125I]⁻ release from [125I]rT3 increased in a time-dependent manner up to 180 min and was almost linear during the initial 120 min. Therefore, an incubation time of 120 min was employed for the subsequent experiments. The release reaction was also temperature-dependent and was maximal at 37 °C. [125I]⁻ release was minimal in the absence of DTT, demonstrating that the reaction was dependent on an enzymatic process.

Cells were further cultured in DMEM in the presence of 15% FBS with 10⁻⁸ M nicotine for up
to 18 h. Kinetic analysis was performed with a Lineweaver-Burk plot. The apparent $K_m$ and $V_{max}$ values for $rT_3$ after 9 h were 3.9 nM and 17 fmol of $I^-$ released/mg protein/h (Fig. 1).

The D2 activity in cells cultured in the presence of $10^{-8}$ M nicotine increased, and reached a peak after 9 h. Thereafter, it decreased by 18 h of incubation, to approximately 50% of that at 9 h (Fig. 2A).

Figure 2B shows the stimulatory effect of nicotine on D2 activity in mixed glial cell cultures. The basal D2 activity was $1.9 \pm 0.2$ fmol of $I^-$ released/mg protein/h. When cells were cultured in the presence of $10^{-12}$, $10^{-11}$, $2 \times 10^{-11}$, $10^{-10}$, and $10^{-9}$ M of nicotine for 9 h, D2 activity increased significantly approximately 2.2-, 2.4, 3.5- and 2.9-fold, respectively, compared with the basal level. But the addition $10^{-12}$ M of nicotine did not increase D2 activity significantly, compared with the basal level.

**Effect of mecamylamine on D2 activity stimulated by nicotine**

When mixed glial cells were cultured in the presence of $10^{-10}$ M nicotine and $10^{-6}$ M mecamylamine, an antagonist of nicotine in the CNS, the nicotine-induced increase in D2 activity was completely abolished to the basal level. The addition of $10^{-6}$ M mecamylamine alone did not affect D2 activity (Fig. 3).

**Discussion**

The CNS is composed of three type of cells, neurons, astrocytes, and oligodendrocytes. Astrocytes not only provide physical support for neurons, but also play an important role in neuronal function [15]. Although astrocytes contain enzymes for both major deiodination pathways [16], the mechanisms regulating D2 activity in the brain remain to be clarified. We found D2 activity in mixed glial cells containing astrocytes and oligodendrocytes [17]. Some agents are reported to increase D2 activity; forskolin, chlorella toxin, TSH, isoproterenol, norepinephrine, the antidepressant desipramine, fluoxetine and dibutyryl cAMP [10, 18-21]. To analyze some factors that regulate D2 activity in the CNS, we used cultures of mixed glial cells and previously reported the stimulatory effect of cyclic guanosine 3',5'-monophosphatc (cGMP) on D2 activity [17].

Acetylcholine is a neurotransmitter in the mammalian CNS and exerts its action on two types of cholinergic receptors, the muscarinic and nicotinic acetylcholine receptors [22]. Astrocytes, as well as neurons, possess both muscarinic and nicotinic receptors [23, 24]. Nicotine produces hyperpolarization of the membrane potential of astrocytes in the rat brainstem and spinal cord, and mecamylamine blocks the stimulatory effect of nicotine [24]. Freund et al. [25] demonstrated that nicotine can exert its electrophysiological effects via a subclass of nicotinic acetylcholine receptors that is neither neuromuscular nor ganglionic in the classical sense and that these brain nicotinic receptors are sensitive to mecamylamine, but not to hexamethonium, α-bungarotoxin, or D-tubocurarine. Colzani et al. [26] reported that nicotine infusion had no effect on serum $T_4$, $T_3$ or...
TSH or liver and kidney D1 activity, but it has not been demonstrated whether nicotine has any effect on brain D2 activity.

We have found that nicotine stimulated D2 activity at concentrations as low as $10^{-10}$ M, and that its stimulatory effect was completely blocked by mecamylamine, which binds to this specific subclass of nicotinic acetylcholine receptors. After smoking, because the serum nicotine concentration may increase to $10^{-5}$-$10^{-6}$ M [27], it seems possible that smoking can actually affect D2 activity in vivo. Moreover, the fact that the addition of $10^{-8}$ M carbamylcholine increased D2 activity to approximately 1.9-fold the basal level, although not significant (data not shown) supports the hypothesis that acetylcholine receptors convey the nicotine effect on D2 activity.

Our findings therefore indicate that nicotine increases D2 activity in rat brain glial cells via nicotinic acetylcholine receptors, and that nicotine or acetylcholine and smoking may have the potential to influence brain function by altering thyroid hormone metabolism. Nevertheless, further studies are required to obtain a clearer understanding of the interactions between acetylcholine and thyroid hormone metabolism in the CNS.
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References

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