

## NICOTINIC ACETYLCHOLINE RECEPTOR ANTAGONISTIC PROPERTY OF THE SELECTIVE DOPAMINE UPTAKE INHIBITOR, GBR-12909 IN RAT HIPPOCAMPAL SLICES

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**Abstract**—Previously we found that inhibitors of noradrenaline (NA) and/or 5-HT reuptake are able to inhibit neuronal nicotinic acetylcholine receptors (nAChRs) in the CNS most probably by a channel blocker-type mechanism. The aim of our study was to clarify whether selective dopamine uptake inhibitors also possess this property, therefore we investigated the effect of GBR-12909 on the nicotine-evoked release of [<sup>3</sup>H]NA from rat hippocampal slices. GBR-12909, similar to selective NA and 5-HT uptake blockers, inhibited the nicotine-evoked release with an IC<sub>50</sub> of 2.32 μM. The ability of monoamine uptake blockers to inhibit nicotine-evoked [<sup>3</sup>H]NA release (IC<sub>50</sub>) and NA reuptake (K<sub>i</sub>) showed no correlation, indicating that the NA uptake system is not involved in the inhibition of the response to nicotine. Previously we have shown in whole cell patch clamp experiments, that GBR-12909, depending on the stimulation pattern, inhibits Na<sup>+</sup>-currents with an IC<sub>50</sub> in the 6–35 μM concentration range [Mike A, Karoly R, Vizi ES, Kiss JP (2003) Inhibitory effect of the DA uptake blocker GBR-12909 on sodium channels of hippocampal neurons. *Neuroreport* 14:1945–1949]. To study whether the inhibition of Na<sup>+</sup>-channels is involved in the action of GBR-12909 on the nicotine-evoked [<sup>3</sup>H]NA release, we compared the effect of GBR-12909 and the Na<sup>+</sup>-channel blocker tetrodotoxin (TTX) on the electrical stimulation- and nicotine-evoked response. TTX prevented the release of [<sup>3</sup>H]NA induced by both types of stimulation, whereas GBR-12909 inhibited only the nicotine-induced response, indicating that under our experimental conditions the target of GBR-12909 is not the Na<sup>+</sup>-channel. These data indicate that the selective DA uptake inhibitor GBR-12909 is able to inhibit nAChRs, that is, the nAChR antagonistic property of monoamine uptake inhibitors is independent of their selectivity. The fact that monoamine uptake inhibitors with different chemical structure and selectivity are able to inhibit nAChRs may reveal some common properties of nicotinic receptors and monoamine uptake carriers. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words:** nicotinic acetylcholine receptors, monoamine uptake systems, selective dopamine uptake inhibitors, GBR-12909.

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**Abbreviations:** AUC, area under the curve; DA, dopamine; DMPP, dimethylphenylpiperazinium; FR, fractional release; NA, noradrenaline; nAChR, nicotinic acetylcholine receptor; nFR, normalized fractional release; SSRIs, selective serotonin reuptake inhibitors; TTX, tetrodotoxin.

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It has been proposed that the beneficial effect of nicotine on cognitive functions is partly mediated by the stimulation of monoaminergic neurotransmission in the CNS (Lee and Ma, 1995; Harley, 2004; Srikumar et al., 2006). To investigate this interaction previously, we studied the effect of nicotinic agonists on the noradrenaline (NA) release from rat hippocampal slices (Vizi et al., 1995; Sershen et al., 1997). We have shown that classical nicotinic agonists (nicotine, epibatidine, cytosine, anatoxin-A) enhance NA release through the stimulation of nicotinic acetylcholine receptors (nAChRs), whereas dimethylphenylpiperazinium (DMPP) induces NA release via two distinct mechanisms, exocytosis induced by the stimulation of nAChRs and carrier-mediated release evoked by the reversal of neuronal reuptake (Kiss et al., 1997, 2001; Szasz et al., 2005). During these experiments we used monoamine uptake inhibitors to prove the involvement of transporters in the response to DMPP, and unexpectedly we found that desipramine, nisoxetine and nomifensine are able to inhibit not only the carrier-mediated NA efflux but also the nAChR-mediated release (Kiss et al., 1997). In subsequent studies we extended the investigation to other monoamine uptake inhibitors (such as the selective serotonin reuptake inhibitors (SSRIs) fluoxetine and citalopram or the major drug of abuse, cocaine), and proved that all of these compounds are able to inhibit nAChRs in the low micromolar concentration range (Hennings et al., 1997, 1999). These findings suggested that the nAChR antagonistic property might be a general feature of monoamine uptake inhibitors. Nevertheless, in these works we studied compounds which are selective NA (desipramine, nisoxetine) or 5-HT inhibitors (fluoxetine, citalopram), or mixed dopamine (DA)/NA inhibitors (nomifensine, cocaine) but selective DA uptake inhibitors were not tested. The aim of the present study was to investigate whether these latter compounds share the nAChR antagonism with other uptake blockers, therefore we studied the effect of a selective DA uptake inhibitor, GBR-12909 on nicotine-evoked NA release from rat hippocampal slices and attempted to identify the mechanism involved in the action of GBR-12909.

### EXPERIMENTAL PROCEDURES

#### Nicotine-evoked [<sup>3</sup>H]NA release from rat hippocampal slices

All experimental procedures were approved by the local ethics committee and were carried out in accordance with the European Communities Council Directive (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of

animals used. The applied method was described earlier (Henings et al., 1999). In brief, male Wistar rats (weight 150–200 g) were killed by decapitation and the brain was rapidly removed and immediately placed into ice-cold Krebs solution (composition in mM: NaCl 113, KCl 4.7, MgSO<sub>4</sub> 1.2, CaCl<sub>2</sub> 2.5, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.2, glucose 115, Na<sub>2</sub>EDTA 0.3 and ascorbic acid 0.03) continuously gassed with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The hippocampi were prepared and sliced into 0.4 mm sections by a McIlwain chopper (Stoelting, Wood Dale, IL, USA). Slices were dissected by shaking and washed with 5 ml Krebs solution and loaded for 45 min with [<sup>3</sup>H]NA (L-7,8-[<sup>3</sup>H]NA, 37 MBq, 30–50 Ci/mmol, Amersham, Budapest, Hungary) at a concentration of 10 μCi in 1 ml of Krebs solution. After the incubation slices were washed three times with 10 ml of ice-cold Krebs solution, they were transferred into a four-channel microvolume perfusion system with an internal volume of 100 μl (Vizi et al., 1985). Four slices were put into each chamber. The temperature inside the chambers was kept at 37 °C. The preparation was superfused with Krebs solution at a rate of 0.5 ml/min for 60 min (preperfusion period), and the effluent was discarded. Subsequently 12 3-min fractions were collected. Nicotine at a concentration of 100 μM was added from the 4th sample; the DA uptake blocker GBR-12909 or mecamylamine was present in the medium from the beginning of the collection period. At the end of the experiments, slices were removed from the chamber and mechanically homogenized in 5 ml of 10% trichloroacetic acid. A 0.5 ml aliquot of the supernatant was added to 2 ml of scintillation cocktail (Ultima Gold, Packard, Downers Grove, IL, USA). Tritium was measured with a Packard 1900 TR liquid scintillation counter using an internal standard. Radioactivity was expressed in terms of disintegration per second per gram of tissue (Bq/g). The fractional release (FR) was expressed in terms of the percentage of tritium present in the tissue at the beginning of a sample collection period.

#### Electrical stimulation-evoked [<sup>3</sup>H]NA release from rat hippocampal slices

Slices were prepared as described above. After the preperfusion period 19 3-min samples were collected. Electrical stimulation (20 V, 2 Hz, 1 ms, 360 impulses) was applied during collection of the third (S<sub>1</sub>) and the thirteenth sample (S<sub>2</sub>). Drugs were administered from collection of the tenth sample onward. The tritium content of the samples was determined as described above.

#### Statistical analysis

FR data were normalized to decrease variance between subjects. The average of three fractions before treatment was taken as 100%, and all fractions were expressed relative to this value as normalized fractional release (nFR) data. The nFR data were analyzed using an area under the curve (AUC) method. The average basal FR data of different treatment groups were not significantly different. The response to nicotine was calculated as the surplus release over the basal efflux. Inhibition values were determined in a counterbalanced manner. In every experiment, we used three different concentrations of the tested drug and a control (nicotine alone) that provided the maximal effect (100%). The inhibitory effect of any drug was expressed relative to this control. Values are mean ± S.E.M. of four to six independent experiments. Dose-response curves were constructed separately for each drug. Comparison of areas was performed by one-way ANOVA followed by Dunnett's test,  $P < 0.05$  was considered significant. IC<sub>50</sub> values were calculated by a nonlinear regression (GraphPad Prism program, GraphPad, San Diego, CA, USA).

In experiments using electrical stimulation, the AUC for S<sub>1</sub> and S<sub>2</sub> was calculated from FR data and inhibition values were determined from the ratio of S<sub>2</sub>/S<sub>1</sub> values (S<sub>2</sub>/S<sub>1</sub> in the presence of drug/S<sub>2</sub>/S<sub>1</sub> in the absence of drug; counterbalanced design, every drug effect was compared with the corresponding control value).

Statistical analysis of the inhibition values was performed using one-way ANOVA followed by Dunnett's *t*-test, as mentioned above.

When the correlation was investigated between the inhibition of nicotine-evoked NA release (IC<sub>50</sub>) and the inhibition of NA uptake (K<sub>t</sub>), the Pearson's correlation analysis was used, and the correlation coefficient (*r*) and slope was determined.

#### Materials

Nicotine bitartrate was obtained from Sigma (St. Louis, MO, USA). GBR-12909, mecamylamine, and tetrodotoxin (TTX) were purchased from Sigma-Aldrich (Budapest, Hungary). Drugs were dissolved in Krebs solution and all other chemicals were of analytical grade.

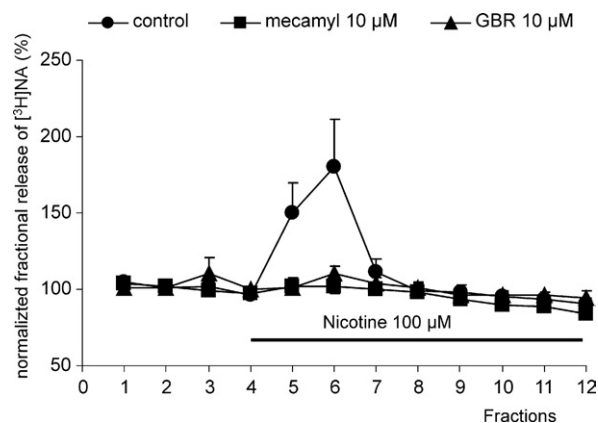
## RESULTS

#### Nicotine-evoked release of [<sup>3</sup>H]NA from rat hippocampal slices

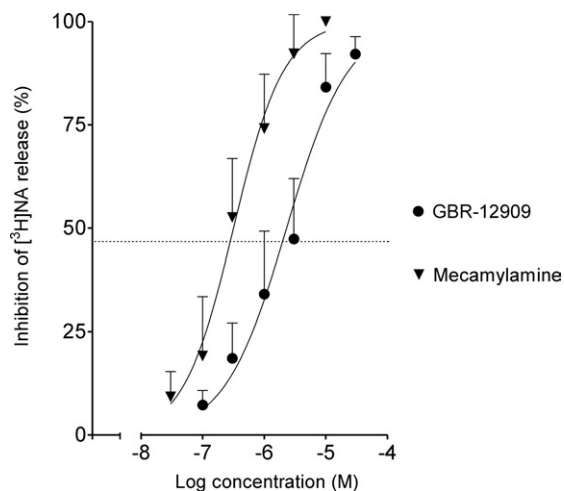
After a 45-min loading with [<sup>3</sup>H]NA followed by a 60 min preperfusion period, the slices contained 3786 ± 299 Bq/mg (mean ± S.E.M.,  $n = 38$ ) radioactivity. Perfusion of nicotine at a concentration of 100 μM produced an excess release over the basal efflux of [<sup>3</sup>H]NA (AUC<sub>4–10</sub> = 299 ± 40%,  $n = 20$ ). The response was transient and the release returned to the baseline within four fractions despite of the presence of nicotine in the solution (Fig. 1). The nicotine-evoked [<sup>3</sup>H]NA-release was completely blocked by the noncompetitive nicotinic antagonist mecamylamine at a concentration of 10 μM, indicating that the response was mediated by nAChRs. The response to nicotine was also sensitive to the selective DA uptake inhibitor GBR-12909, which produced an almost complete inhibition at a concentration of 10 μM (Fig. 1).

#### Effect of GBR-12909 on the nicotine-evoked release of [<sup>3</sup>H]NA from rat hippocampal slices

The nicotine-evoked release of [<sup>3</sup>H]NA was inhibited in a dose-dependent manner by mecamylamine with an IC<sub>50</sub>



**Fig. 1.** Effect of nicotine and GBR-12909 on the basal release of [<sup>3</sup>H]NA from rat hippocampal slices. Nicotine (100 μM) was applied from the 4th 3-min fraction, the non-competitive nicotinic antagonist mecamylamine (mecamyl) or the selective DA uptake inhibitor GBR-12909 (GBR) was present from the beginning of the collection period. The AUC was calculated as the surplus release over a theoretical baseline. Comparison of areas was performed by one-way ANOVA followed by Dunnett's test,  $P < 0.05$  was considered significant.



**Fig. 2.** Dose-response curve of GBR-12909 and mecamylamine on the nicotine-evoked [ $^3\text{H}$ ]NA release from rat hippocampal slices. Each tested drug was present in the Krebs solution from the beginning of the collection period, nicotine was administered from the 4th fraction onward. Inhibition values were calculated as the ratio of the AUC in the presence and absence of each drug, 50% inhibition is represented by the dotted line.  $\text{IC}_{50}$  values were determined by nonlinear regression.

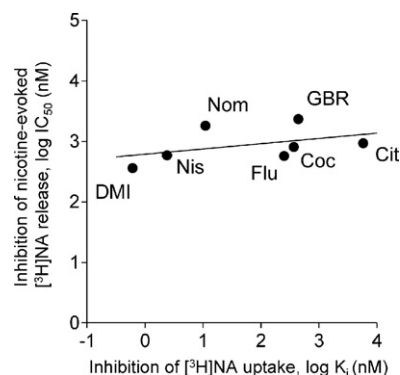
value of  $0.32 \mu\text{M}$ . GBR-12909 produced a similar dose-dependent inhibition in the concentration range of  $0.1\text{--}30 \mu\text{M}$  (Fig. 2). The  $\text{IC}_{50}$  value of GBR-12909 was  $2.32 \mu\text{M}$ , only an order of magnitude lower than that of mecamylamine.

### Correlation between the inhibition of nicotine-evoked [ $^3\text{H}$ ]NA release and the inhibition of NA uptake

In our previous experiments, the  $\text{IC}_{50}$  values of selective and nonselective monoamine uptake blockers ranged between  $0.36 \mu\text{M}$  (desipramine) and  $1.84 \mu\text{M}$  (nomifensine) (Hennings et al., 1997, 1999). These data were obtained under identical experimental conditions as the data for the inhibitory constant of GBR-12909. To explore a possible relationship between the observed nAChR antagonist effect and the ability to block NA uptake, the logarithms of the calculated  $\text{IC}_{50}$  values of each monoamine blocker were plotted against the logarithms of the  $K_i$  values for the NA uptake transporter and the coefficient of correlation ( $r$ ) was determined (Fig. 3).  $K_i$  values were taken from the literature (Hytel, 1982; Andersen, 1989; Bolden-Watson and Richelson, 1993; Wong et al., 1995). The Pearson's correlation analysis showed no correlation between the two variables (slope=0.086,  $r=0.43$ ,  $P=0.33$ , not significantly different from zero).

### Comparison of the inhibitory effect of TTX and GBR-12909 on the nicotine- and electrical stimulation-evoked release of [ $^3\text{H}$ ]NA from rat hippocampal slices

The effect of GBR-12909 and the  $\text{Na}^+$ -channel blocker TTX on the nicotine- and electrical stimulation-evoked release of NA was compared, so as to test the possible involvement of  $\text{Na}^+$  channels in the inhibitory action of

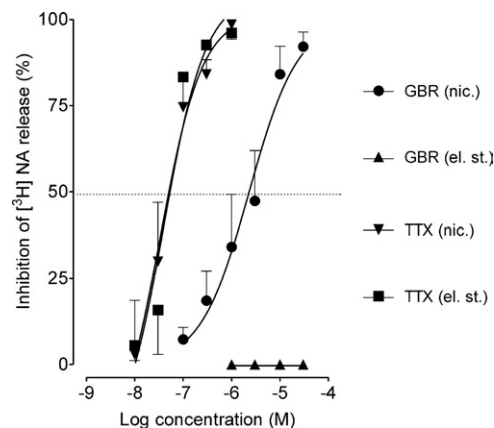


**Fig. 3.** Correlation between the inhibitory effect of monoamine uptake blockers on synaptosomal [ $^3\text{H}$ ]NA uptake and nicotine-evoked [ $^3\text{H}$ ]NA release. The  $K_i$  values characterizing uptake inhibition were taken from the literature (Hytel, 1982; Andersen, 1989; Bolden-Watson and Richelson, 1993; Wong et al., 1995), the  $\text{IC}_{50}$  values characterizing the inhibition of nicotine-evoked [ $^3\text{H}$ ]NA release were determined in our recent and previous (Hennings et al., 1997, 1999) studies under identical experimental conditions. The Pearson's correlation analysis was used to investigate the linear relationship between the two variables and the correlation coefficient ( $r$ ) was determined, the level of significance was set at  $P < 0.05$ . Abbreviations: DMI: desipramine; Nis; nisoxetine; Nom: nomifensine; Cit: citalopram; Coc: cocaine; Flu: fluoxetine; GBR: GBR-12909.

GBR-12909. TTX blocked both the nicotine- and the electrical stimulation-evoked release with the same efficacy ( $\text{IC}_{50}$  was  $33 \text{ nM}$  and  $39 \text{ nM}$ , respectively) (Fig. 4). In contrast, GBR-12909 blocked only the nicotine-evoked release ( $\text{IC}_{50}=2.32 \mu\text{M}$ ), whereas the electrical stimulation-evoked release was not inhibited at all.

## DISCUSSION

The major aim of this study was to investigate whether the selective inhibitors of neuronal DA uptake possess similar nAChR antagonistic property as do the previously studied NA and 5-HT uptake inhibitor compounds. We have chosen GBR-12909 as a representative of highly selective DA uptake



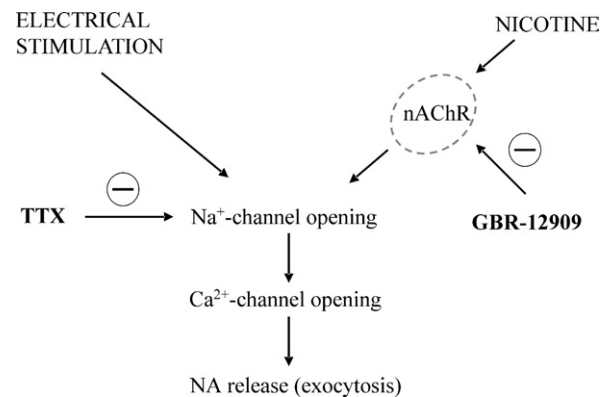
**Fig. 4.** Comparison of the effect of GBR-12909 (GBR) and TTX on the nicotine (nic.)- and electrical stimulation (el. st.)-evoked release of [ $^3\text{H}$ ]NA from rat hippocampal slices. Fifty percent inhibition is represented by the dotted line.  $\text{IC}_{50}$  values were determined by nonlinear regression.

inhibitors, because this compound inhibits DA uptake into striatal synaptosomes with a  $K_i$  of 1 nM, whereas its inhibitory effect on NA and 5-HT uptake is much weaker; the  $K_i$  values are 440 and 170 nM, respectively (Andersen, 1989). We have studied the effect of GBR-12909 on the nicotine-evoked [ $^3$ H]NA release from rat hippocampal slices, using the method proven to be very sensitive for detection of the inhibitory effect on nAChRs (Hennings et al., 1997, 1999), furthermore the same experimental protocol assured a reliable comparison with previous results. Under these conditions, GBR-12909 very effectively inhibited the nicotine-evoked [ $^3$ H]NA release. The inhibitory effect of the potent nAChR antagonist mecamylamine was only seven times stronger (Fig. 2).

Nevertheless, the inhibition of the response to nicotine can be achieved not only by a direct effect on nAChRs, but also by a number of indirect mechanisms. It has been suggested that the action of nicotine on NA release may require an intact NA uptake process (Su and Bevan, 1970), that is, a functional antinicotinic action of uptake blockers could also be mediated through the transporter. If this is the case, however, a correlation should exist between the inhibitory effects on NA uptake and nicotine-evoked NA release. To test this hypothesis, we compared the  $IC_{50}$  values of GBR-12909 and a number of other monoamine uptake inhibitors investigated previously (Hennings et al., 1997, 1999) with the inhibitory effect of these compounds on NA uptake. Since we used identical experimental protocols, merging data obtained in different studies did not cause any problem in the interpretation of results. The  $K_i$  values of monoamine uptake inhibitors on NA uptake into cortical synaptosomes were taken from the literature (Hytel, 1982; Andersen, 1989; Bolden-Watson and Richelson, 1993; Wong et al., 1995). The statistical analysis showed no correlation between  $K_i$  and  $IC_{50}$  values (Fig. 3), suggesting that the NA uptake system is not involved in the inhibitory effect of GBR-12909 and other monoamine uptake blockers on the nicotine-evoked NA release. This conclusion is supported by our previous observation that nomifensine at a concentration of 1  $\mu$ M effectively blocked the carrier-mediated component of DMPP-evoked NA release but did not affect the nAChR-mediated component (Kiss et al., 1997), indicating that the actions on the transporter and on the nAChR are independent phenomena.

Another possible target for the action of GBR-12909 could be the voltage dependent  $Na^+$ -channel. We have provided evidence that the nicotinic agonist-evoked NA release from rat hippocampal slices is TTX-sensitive (Kiss et al., 1997; Sershen et al., 1997), that is, the activation of  $Na^+$ -channels is a prerequisite for the response. In addition, it has been shown that GBR-12909 binds with high affinity to the  $Na^+$ -channel (Andersen, 1989); thus, the involvement of  $Na^+$ -channels in the action of GBR-12909 had to be investigated. In previous whole cell patch clamp studies (Mike et al., 2003, 2004), we demonstrated that GBR-12909 inhibits  $Na^+$ -currents evoked by trains of depolarizing pulses with an  $IC_{50}$  value of 6.3  $\mu$ M. A weaker inhibition ( $IC_{50}$  = 17–35  $\mu$ M) could be observed when currents were evoked by either single pulse depolarization or

from hyperpolarized holding membrane potential. These data indicated that the extent of inhibition caused by GBR-12909 depends on the physiological activity pattern of neurons. Since it is hard to determine what kind of activity pattern is generated by nicotine in our slice preparation, the involvement of  $Na^+$ -channels could not be excluded unanimously in electrophysiological studies. In the next series of experiments, therefore we compared the ability of GBR-12909 to inhibit nicotine- and electrical stimulation-evoked release of [ $^3$ H]NA release from rat hippocampal slices. Our data using the  $Na^+$ -channel blocker TTX showed that the inhibition of  $Na^+$ -channels prevents NA release with the same efficacy independently of the method of stimulation, indicating that  $Na^+$ -channels play a similarly important role in both electrical stimulation- and nicotine-evoked release. However, in our experiments, GBR-12909 inhibited only the nicotine-evoked release, but had no effect on the electrical stimulation-evoked response (Fig. 4), indicating that  $Na^+$ -channels cannot be involved in the action of the DA uptake inhibitor under our experimental conditions. The first step in the nicotine-evoked NA release is the activation of nAChRs by the agonist, which opens the ligand-gated ion channel and allows the influx of cations into the cell. This results in an initial depolarization which opens the  $Na^+$ -channels and further depolarizes the neurons. Finally the voltage dependent calcium channels are also activated, which leads to the exocytic release of NA (Fig. 5). It is clear that from the activation of  $Na^+$ -channels the electrical stimulation- and nicotine-evoked release use a common pathway. The selective inhibition of nicotine-evoked release, therefore, excludes the involvement of  $Na^+$ -channels and any downstream events in the action of GBR-12909. Since the previous step before the



**Fig. 5.** Differences in the mechanism of inhibition by GBR-12909 and TTX. The stimulation by nicotine activates nAChRs, which leads to an initial depolarization and activation of voltage dependent  $Na^+$ -channels. The further membrane depolarization activates voltage dependent  $Ca^{2+}$ -channels, which results in exocytic release of NA. The electrical stimulation directly opens  $Na^+$ -channels, and from this point on, the downstream events are identical. TTX blocks NA release induced by both stimulations, since the  $Na^+$ -channel is involved in both pathways. The fact that GBR-12909 selectively inhibits the nicotine-evoked NA release indicates that the target of this compound must precede the  $Na^+$ -channels. Since the previous step before the activation of  $Na^+$ -channels is the nAChR-mediated depolarization of cell membranes, our data suggest that the primary target of GBR-12909 is the nAChR.

activation of Na<sup>+</sup>-channels is the nAChR-mediated depolarization of cell membranes, our data suggest that the primary target of GBR-12909 is the nAChR.

Although the nature of nicotinic antagonism was not investigated, based on the literature we can propose a possible mechanism of action. It has been shown that the tricyclic desipramine (Aronstam, 1981), cocaine (Lerner-Marmarosh et al., 1995) and the SSRI fluoxetine (Garcia-Colunga et al., 1997; Maggi et al., 1998) can bind into the pore region of nAChRs, and exert their inhibitory effect on nAChRs by a channel blocker-type mechanism. Another group reported that four further monoamine uptake inhibitor antidepressants, the SSRI sertraline and paroxetine, and the serotonin and noradrenaline reuptake inhibitor (SNRI) nefazodone and venlafaxine inhibit nAChRs in a non-competitive manner (Fryer and Lukas, 1999a) which is characteristic of channel blocker-type antagonists. In the last decade, a number of independent research groups reported similar observation in connection with other monoamine uptake inhibitor-type antidepressants, such as imipramine, amitriptyline, bupropion or reboxetine (Izaguirre et al., 1997; Park et al., 1998; Fryer and Lukas, 1999b; Slemmer et al., 2000; Miller et al., 2002). These results indicate that monoamine uptake blockers with different chemical structures and selectivity interact directly with the ion channel of nAChRs, and probably other monoamine uptake blockers (including GBR-12909) share the same mechanism of action, that is, they bind into the ion channel of nAChRs and act as non-competitive channel blocker-type nicotinic antagonists.

Our data provide further support for the idea that the nAChR antagonism is a general feature of monoamine uptake inhibitors. What can be the explanation for this unexpected phenomenon? Accumulating data indicate that monoamine transporters possess channel properties (Larsson et al., 1996; Lester et al., 1996; Sonders and Amara, 1996; Sonders et al., 1997). It has been shown that the neuronal reuptake of NA is associated with an ionic conductance not stoichiometrically coupled to the transport process, and these currents could be blocked by cocaine and antidepressants (Galli et al., 1995). Recently it has been demonstrated that DA transporters depolarize neurons by a channel mechanism, and the DA-induced channel activity is blocked by selective DAT inhibitors and disappears in DA transporter knockout animals (Carvelli et al., 2004). These data support the idea that functionally important channel-like structures exist in uptake systems (DeFelice and Blakely, 1996; Sulzer and Galli, 2003), and the inhibition of these channels in transporters is a common property of monoamine uptake blockers. If we assume some structural similarity between channels in transporters and the pore of nAChRs, the nicotinic antagonist property of monoamine uptake inhibitors can be easily explained. The model we propose predicts that channel blocker-type nicotinic antagonists should block the monoamine uptake. Indeed, in preliminary synaptosomal uptake studies, we provided evidence that the non-competitive nAChR antagonist mecamylamine, which binds into the pore of the receptor, is able to block monoamine uptake (Kiss,

J.P., unpublished observations). These data seem to support our assumption nevertheless, further investigation is required to elucidate the answer to this question.

## CONCLUSION

In conclusion, we provided evidence that the selective DA uptake inhibitor GBR-12909 is able to block nAChRs in the CNS, which suggests that the nAChR antagonistic property of monoamine uptake inhibitors is independent of their selectivity and might be a general feature of these drugs. Our finding provides pharmacological evidence for the channel properties of monoamine uptake systems and might shed light on some structural similarities between monoamine transporters and nAChRs.

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