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Research Report

Nicotinic receptor inactivation after acute and repeated in vivo nicotine exposures in rats

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ABSTRACT

Nicotine tolerance is often accompanied by an upregulation of brain area nicotinic acetylcholine receptors (nAChRs) in both animal and human subjects. This upregulation has been hypothesized to result from repeated or prolonged exposures of these receptors to nicotine. To explore this further, this study examined the level of nAChR desensitization following acute and repeated nicotine administration in the male Lewis rat. Nicotine-stimulated $^{86}Rb^{+}$ efflux was measured in synaptosomes prepared from the frontal cortex, hippocampus, striatum, and thalamus. Analysis of receptor functionality was achieved by calculating area-under-the-curve (AUC) for nicotine-induced fractional $^{86}Rb^{+}$ efflux. Nicotine-stimulated $^{86}Rb^{+}$ efflux from all brain regions was significantly less in rats that received an acute injection of 0.4 mg/kg nicotine (s.c.) 15 min prior to dissection compared to control rats. This decrease in nAChR functional status was also observed in rats treated with 1 day or 14 days of twice-daily nicotine administration. These results are consistent with the concept that acute exposure to nicotine induces rapid desensitization of nAChRs. In addition, following repeated exposure to nicotine, nAChRs did not become tolerant to the loss in receptor function that occurs after an initial nicotine administration. Overall, these data suggest that neuronal adaptations underlying nicotine tolerance may begin upon initial exposure then persist following repeated exposures.

1. Introduction

Nicotine's ability to induce receptor desensitization has been studied extensively using in vitro, as well as ex vivo and in vivo models (Damaj et al., 1996; Hulihan-Giblin et al., 1990a, 1990b; Lukas, 1991; Marks et al., 1994; Sharp and Beyer, 1986; Stolerman et al., 1973). Repeated administration of agonists at most types of receptors induces a receptor downregulation, whereas repeated nicotine administration appears to lead to an upregulation of nAChRs (Fenster et al., 1999; Marks et al., 1983; Schwartz and Kellar, 1985). It has been suggested that nicotine's effects on distinct neural pathways account for the differential behavioral effects associated with nicotine (Stolerman, 1991) and may also account for nicotine-induced tolerance, dependence, and withdrawal symptoms associated with nicotine addiction.

In addition to its initial effect of increasing ion conductance through the nAChR's ligand-gated channel, continued exposure to nicotine leads to loss of nAChR function ("reversible desensitization") and even more prolonged exposure to nicotine lead to a persistent loss of function ("persistent inactivation"), accompanied by a paradoxical increase in

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nAChR binding (Collins and Marks, 1996; Dani and Heinemann, 1996; Lester and Dani, 1994; Lukas, 1991). This increase in nAChR-like ligand binding sites appears to involve internal rather than cell-surface binding sites and therefore may have little or no physiological relevance (Ke et al., 1998; Whiteaker et al., 1998). The present study evaluated brain area synaptosomal nicotine-induced release of $^{86}Rb^{+}$ to determine potential differences in receptor function following various dosing schedules in the male Lewis rat.

2. Results

Representative fractional release tracings from $^{86}Rb^{+}$ efflux assays performed on tissue obtained from control, acutely or repeatedly nicotine-dosed rats are shown in Fig. 1. The effects of nicotine (10 or 30 μM) stimulation on $^{86}Rb^{+}$ efflux are illustrated in Fig. 2. Significant effects of treatment were observed on 10 μM nicotine-induced $^{86}Rb^{+}$ efflux in the striatum ($F[3,20] = 6.937$, $P = 0.0022$), frontal cortex ($F[3,20] = 3.35$, $P = 0.04$), hippocampus ($F[3,20] = 14.272$, $P < 0.0001$), and thalamus ($F[3,20] = 5.291$, $P = 0.0075$). Post hoc tests revealed significant effects of 1, 2, and 28 doses of 0.4 mg/kg nicotine on 10 μM nicotine-induced $^{86}Rb^{+}$ efflux in all brain regions, compared to control rats ($P < 0.05$), with nicotine-treated rats exhibiting smaller responses to nicotine compared to control rats. No other significant differences were observed.

Significant effects of treatment also were observed on 30 μM nicotine-induced $^{86}Rb^{+}$ efflux in the striatum ($F[3,20] = 11.108$, $P = 0.0002$), frontal cortex ($F[3,20] = 10.458$, $P = 0.0002$), hippocampus ($F[3,20] = 6.173$, $P = 0.0038$), and thalamus ($F[3,20] = 4.670$, $P = 0.0125$). Post hoc tests revealed significant effects of 1, 2, and 28 doses of 0.4 mg/kg nicotine pretreatment compared to control rats on 30 μM nicotine-induced $^{86}Rb^{+}$ efflux in all brain regions ($P < 0.05$), with smaller responses to nicotine being observed with nicotine pretreatment compared to control rats. Significant differences in 30 μM nicotine-induced $^{86}Rb^{+}$ efflux were observed in the saline pre-treated rats as a function of region ($F[3,63] = 5.09$, $P = 0.003$). Post hoc tests revealed significant differences in fractional areas for the striatum and frontal cortex compared to the hippocampus and thalamus ($P < 0.05$), with significantly smaller responses to nicotine being observed in the striatum and frontal cortex.

3. Discussion

The present study demonstrated diminution of nAChR function in the striatum, frontal cortex, hippocampus, and thalamus of rats following various in vivo nicotine pretreatments. Consistent with other reports in the literature (Hulihan-Giblin et al., 1990a, 1990b), the findings from this study suggest that desensitization and/or persistent inactivation of the receptors mediating $^{86}Rb^{+}$ efflux occurs following either

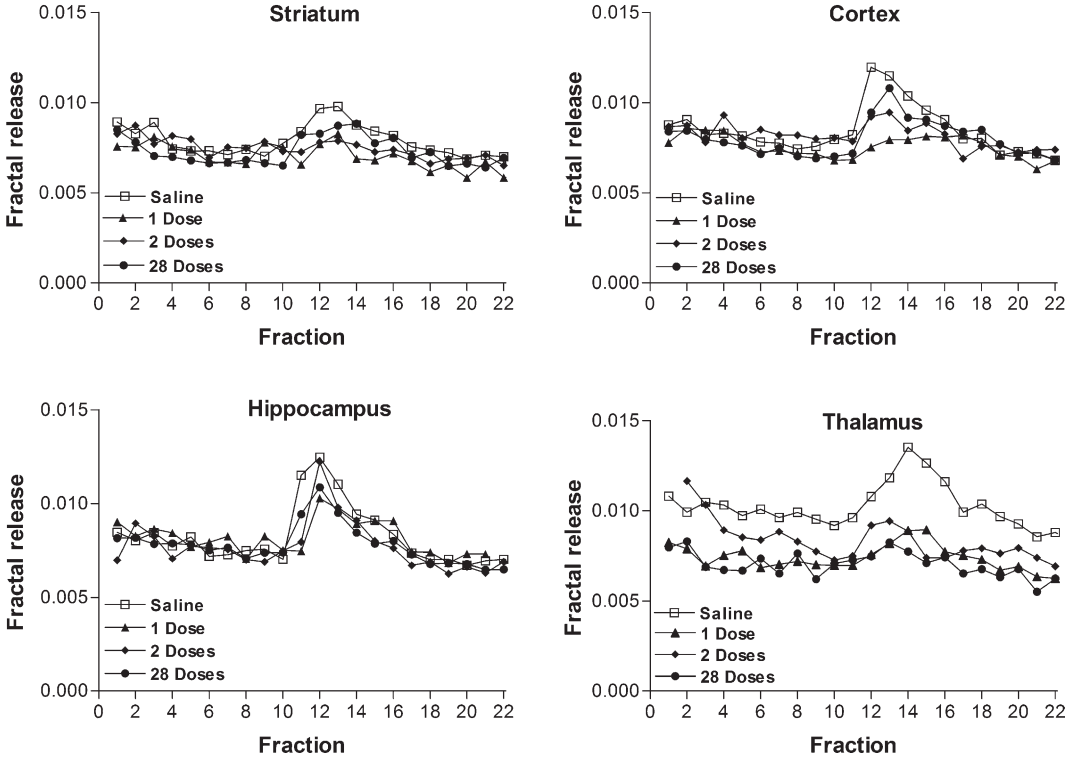

Fig. 1 – Representative data of nicotine (30 μM)-stimulated $^{86}Rb^{+}$ efflux in synaptosomes prepared from frontal cortex, hippocampus, striatum, and thalamus of male Lewis rats from four different treatment groups (saline, acute nicotine, 2 doses of nicotine and 28 doses of nicotine). All nicotine (0.4 mg/kg free base) injections were made subcutaneously. Data are expressed as fractional release (cpm of $^{86}Rb^{+}$ per 12-s fraction divided by total tissue cpm) for each fraction collected.