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Short Communication

Opposing Effects of Clomipramine on [^{125}I]RTI-55 and [^{3}H]N-Methylspiperone Binding in Mouse Striatum: Important Role of Other Factors Than Endogenous Dopamine?

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Neuroreceptor imaging with Positron Emission Tomography (PET) is a potential method for revealing neural interactions in intact animal or human brain. Changes in in vivo binding of dopamine receptors by modifications of GABAnergic, cholinergic as well as dopaminergic systems have been reported (Dewey et al., 1992, 1993a,b). The mechanism for changes in dopamine receptor binding have been thought to be mainly due to competitive inhibition by endogenous dopamine (Dewey et al., 1992, 1993b). However, several reports provide evidence that factors other than endogenous dopamine might influence the rates of association or dissociation of ligand-receptor interactions in vivo, resulting in alterations in apparent receptor binding (Inoue et al., 1992; Kobayashi and Inoue, 1993). It has been previously reported that [^{123}I]-RTI-55 binding in the striatum of rats pretreated with clomipramine was significantly and dose-dependently increased (Fujita et al., 1997), while many kinds of antidepressants including clomipramine decrease both [^{3}H]SCH 23390 and [^{3}H]NMSP binding in the mouse striatum (Suhara et al., 1990). These discrepancies in binding between presynaptic and postsynaptic receptors are difficult to explain in terms of competitive inhibition by endogenous dopamine. As the RTI-55 binding study was performed in rats, but the changes in D_1 and D_2 receptor binding were measured in mice, one possibility for such opposing effects may be due to species differences. In addition, [^{3}H]SCH 23390 and [^{3}H]-NMSP binding in mouse striatum were measured at only one time interval (30 minutes) after tracer injection. In order to confirm and quantify this phenomenon in the same species, the time course of [^{125}I]RTI-55 or [^{3}H]NMSP binding in control or clomipramine pretreated (30 mg/kg, i.p.) mice was determined. Mice were intravenously injected with either 0.1 MBq of [^{125}I]RTI-55 or [^{3}H]NMSP, and decapitated at various time intervals after tracer injection. The brains were quickly removed, dissected into cerebral cortex, striatum, and cerebellum, and weighed. The radioactivity of [^{125}I]RTI-55 or [^{3}H]NMSP in each region was measured, and radioactivity concentrations were expressed as percent injected dose per gram of tissue weight (% dose/g). For both radioligands, the cerebellum was used as a reference region for the estimation of free and nonspecifically bound ligand. The specific binding at each time point was estimated by subtraction of radioactivity in cerebellum from total radioactivity in the region of interest. Figure 1 shows the time course of the [^{3}H]NMSP or [^{125}I]RTI-55 specific binding in control and clomipramine treated mice. Significant decreases in the binding [^{3}H]NMSP were observed in the striatum and cerebral cortex.

Clomipramine caused a significant decrease of [^{125}I]RTI-55 binding in the cerebral cortex. In contrast, increased RTI 55 binding was observed in the striatum. Kinetic analysis using the Patlak Plot revealed a 20% increase of the k_3 value for RTI-55, but a 40% decrease in k_3 for NMSP in the striatum of mice pretreated with clomipramine. The results obtained using the two-compartment model for cortical tracer binding showed that clomipramine decreased 58% of binding potential for [^{3}H]NMSP and 36% of [^{125}I]RTI-55, respectively.

As it is known that the accumulation of [^{3}H]NMSP in the cerebral cortex reflects serotonin S_2 receptor binding, and that clomipramine inhibits serotonin reuptake, the reduction in binding of [^{3}H]NMSP in this region might be due to competitive inhibition by seroto-

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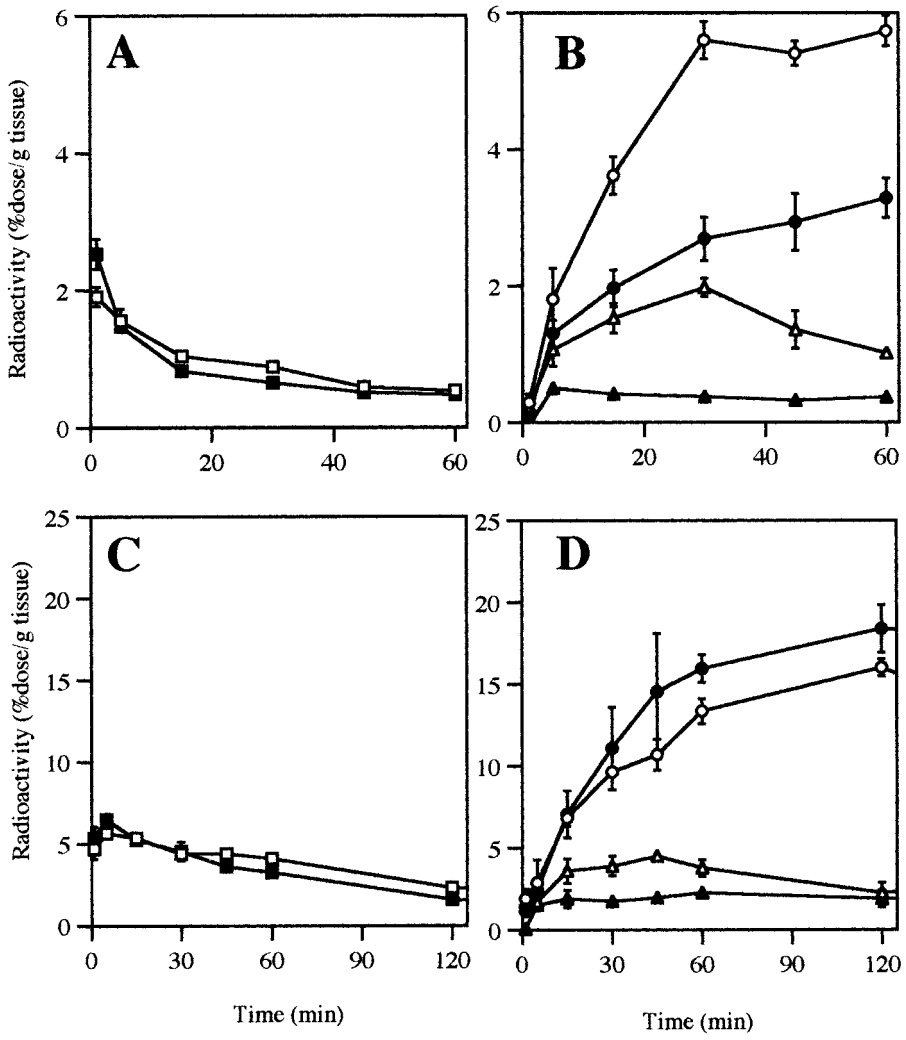

Fig. 1. The time course of [^{3}H]NMSP (**A,B**) or [^{125}I]RTI-55 (**C,D**) specific binding in striatum and cerebral cortex (B,D) or radioactivity in cerebellum (A,C) of control and clomipramine pretreated mice. Control: cerebellum, □; striatum, ○; cerebral cortex, △; clomipramine: cerebellum, ■; striatum, ●; cerebral cortex, ▲.

nin. Clomipramine also inhibits dopamine reuptake in striatum, causing a significant increase in the extracellular dopamine level (Ichikawa and Meltzer, 1995). Therefore, a significant decrease in [^{3}H]NMSP binding in striatum can be partly explained by increased competition from endogenous dopamine. However, it is still possible that other factors may also influence apparent receptor binding in vivo, as we previously proposed (Inoue et al., 1995).

As RTI-55 binds to the serotonin transporter in the cortex (Boja et al., 1992), the reduction of cortical RTI binding might be due to competitive inhibition by clomipramine itself. The most important finding in this experiment is that RTI-55 binding in striatum was significantly increased in clomipramine pretreated mice, which is consistent with the previous observation in rats (Fujita et al., 1997). These findings provide convincing evidence that apparent ligand-receptor binding in vivo can be greatly influenced by factors other than competition by endogenous dopamine. The in vivo dissociation rate constants (k_{off}) for both [^{3}H]NMSP and [^{125}I]RTI-55 are generally regarded as being negligible within 1 or 2 hours post administration. Thus, the apparent binding observed in this study should mainly reflect two components: the bimolecular association rate constant (k_{on}) and the maximum number of binding sites available (B_{max}). As previously reported, no significant changes in the B_{max} of [^{3}H]NMSP binding in striatum were observed by antidepressants pretreatment (Suhara et al., 1990). Thus, it is most likely that clomipramine induced a decrease in the association rate of [^{3}H]NMSP binding in this study. In contrast, an increase in the association rate of RTI binding seems to be the main reason for the increase in RTI binding by clomipramine.

In conclusion, the results of this study cannot be easily explained by a change in endogenous dopamine concentrations. Although endogenous neurotransmitters may exert an influence on radioligand binding, other, as yet unexplained, factors appear to be of significance in the interneuronal modulation of receptor binding in vivo.

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