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Cocaine Receptors: In Vivo Labeling With ^{3}H-(−)Cocaine, ^{3}H-WIN 35,065-2, and ^{3}H-WIN 35,428

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Several cocaine binding sites have been identified in the brain and periphery. In the rodent striatum and in the human basal ganglia the binding is to some aspect of the dopamine transporter. This cocaine binding site has been related to drug self-administration and to cocaine dependence (for references, see Ritz et al., 1987).

Various ligands have been used to label the dopamine transporter in vivo (Aquilonius et al., 1987; Leenders et al., 1988; Kilbourn, 1988; Kuhar et al., 1989). Recently, ^{11}C-cocaine was used in PET scanning studies with primates and humans to label the cocaine receptor in vivo (Fowler et al., 1989). Several cocaine analogs exist that have a higher affinity for the binding site than cocaine and that are less vulnerable to metabolism than cocaine. Two of these compounds are WIN 35,065-2 and WIN 35,428. Both compounds are behaviorally active (Spealman et al., 1977; Ritz et al., 1987) and have been radiolabeled and examined in in vitro binding assays (Madras et al., 1989; Ritz et al., 1989). As expected, they have a higher affinity for the receptor and higher specific to nonspecific binding ratios in vitro. In this study, we compare the binding of ^{3}H-(−)cocaine with these compounds in vivo in the mouse.

^{3}H-(−)cocaine (31.9 Ci/mmol), ^{3}H-WIN 35,065-2 (81.3 Ci/mmol), and ^{3}H-WIN 35, 428 (CFT; 80 Ci/mmol) were purchased from DuPont NEN (Boston, MA). Male CD-1 mice (25–32 gm) were purchased from Charles River Labs (Wilmington, MA). Both radiolabeled compounds and drugs were injected via the tail vein in 0.2 ml saline; drugs were administered 5 min prior to injection of radioactive tracers. The animals were sacrificed by cervical dislocation and the striatum and cerebellum dissected rapidly on ice. The tissues were dissolved with Protosol (DuPont NEN), and radioactivity content per milligram of tissue wet weight was assayed by liquid scintillation spectrometry.

Groups of mice were injected with 2 μCi of radioactive compound as described above, and, after determining radioactivity content of the tissues, we calculated striatal to cerebellar (S/C) ratios of radioactivity. These ratios reflect the relative accumulation of drug in the striatum compared with in the cerebellum and would be greater than 1 if the drugs were preferentially binding to dopamine transporters on dopaminergic terminals, which are highly concentrated in the striatum and absent in the cerebellum.

After injection with ^{3}H-(−)cocaine, S/C ratios were 1.56 ± 0.02 (n = 4; mean ± SEM) at the earliest time examined, 5 min. This corresponded to 394, 139 dpm/gm tissue of striatum and 253,007 dpm/gm tissue of cerebellum. The ratio rapidly declined to near unity by 30 min. By contrast, both ^{3}H-WIN 35,065-2 and ^{3}H-WIN 35,428 had S/C ratios that peaked at about 45 min and were much higher, 3.3 ± 0.08 and 3.94 ±0.12, respectively (Fig. 1). Also, the retention of the latter two compounds was significantly longer than that of (−)cocaine. This corresponds to the observations that these compounds are more potent and longer lasting in behavioral studies (Spealman et al., 1977; D'Mello et al., 1981).

To be sure that the accumulated radioactivity was associated with the cocaine receptors on dopamine transporters, the pharmacological properties of the accumulation were examined. Groups of mice were pretreated with various drugs and injected with radioactive ^{3}H-WIN 35,065-2 as described above. Compounds that have little or no affinity for the transporter did not inhibit accumulation; these include paroxetine, which has a high affinity for serotonin transporters, desipramine, which has high affinity for norepinephrine transporters, and (+)cocaine, which has little affinity for any transporter. By contrast, compounds that have significant affinity for the dopamine transporter blocked the accumulation effectively; these include (−)cocaine, GBR 12909, nomifensine, and mazindol (Fig. 2). The block was dose-related, since lower doses of (−)cocaine, for example, had less of an effect. The observed reduced S/C ratio is due to reduction of radioactivity in the striatum, since the cerebellar content was unchanged. Also, ^{3}H-WIN 35,065-2 binding in vivo was saturable (data not shown). Similar studies are underway with ^{3}H-WIN 35,428.

These data show that both cocaine analogs provide greater and longer lasting specific binding in vivo than (−)cocaine itself. This presumably is due to the higher affinity of these compounds for the binding site and the lower potential for metabolism. The latter is presumably due to the lack of ester bond between the phenyl group and the tropane ring (Fig. 1). This ester link is hydrolyzed in vivo, perhaps by an enzymatic mecha-

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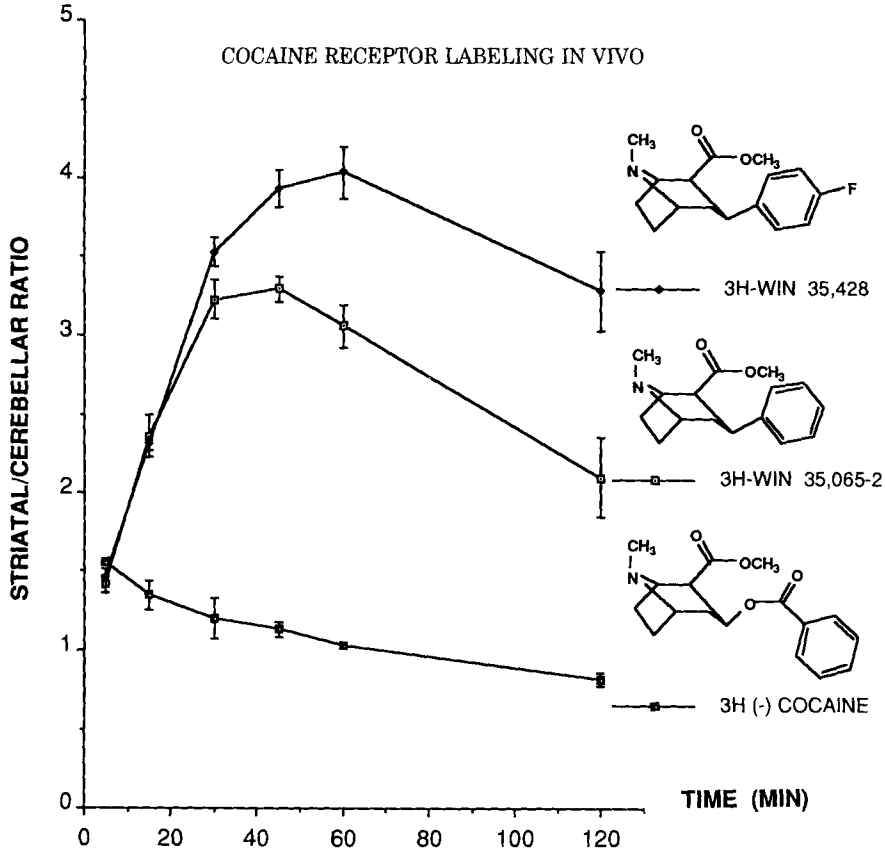

Fig. 1. Time course of striatal to cerebellar ratios for ^{3}H-WIN 35,428; ^{3}H-WIN 35,065-2; and ^{3}H-(−)cocaine. See text for experimental details. Data are expressed as means ± SEM, n = 4. The structures of the compounds are shown next to their respective data.

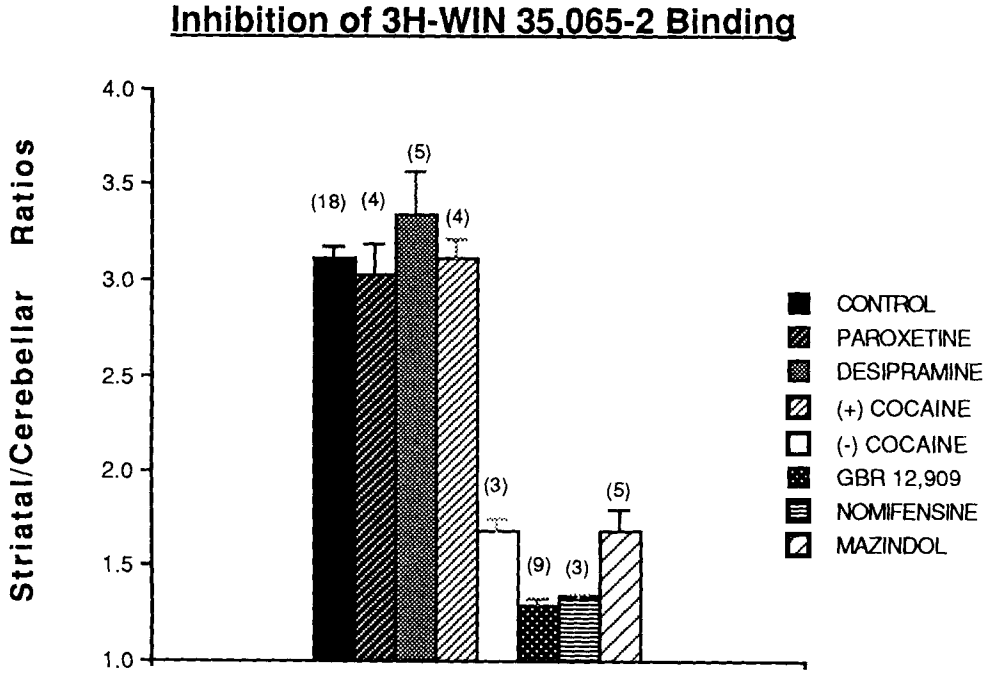

Fig. 2. Effects of various drugs on striatal/cerebellar ratios. Data are means ± SEM. Numbers of animals are shown in parentheses above the bars. See text for details. Drug doses were 10 mg/kg, except for mazindol, which was 1 mg/kg. Animals were sacrificed 30 min after injection of tritiated compounds.

nism (Baselt, 1982). Various studies are being carried out to examine these possibilities.

In summary, the two analogs exhibit improved binding in vivo in that S/C ratios are much higher and of longer duration than are those for cocaine. These results establish the feasibility of a mouse model for in vivo labeling of the cocaine receptor and support the testing of these compounds in PET studies.

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