Pharmacology Biochemistry & Behavior, Vol. 41, pp. 235–238. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Selective Up-Regulation of D-1 Dopamine Receptors Following Chronic Administration of SCH 39166 in Primates

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Received 23 May 1991

DUFFY, R. A., G. KAMINSKA, R. E. CHIPKIN AND R. D. McQUADE. *Selective up-regulation of D-1 dopamine receptors following chronic administration of SCH 39166 in primates.* PHARMACOL BIOCHEM BEHAV **41**(1) 235–238, 1992.—Caudate, putamen and frontal cortex tissues were obtained from rhesus monkeys that had taken part in a toxicology study required by the Food and Drug Administration. These monkeys had received daily oral treatments of SCH 39166 at three different doses (3, 12 and 48 mg/kg) for three consecutive months. Plasma membranes from the caudate and putamen were analyzed for changes in D-1 and D-2 receptor affinity and number using saturation analyses of ^{3}H-SCH 23390 and ^{3}H-spiperone binding, respectively. Saturation studies were performed on membranes from the frontal cortex using ^{3}H-ketanserin to determine if $5HT_2$ receptor number or affinity were affected by chronic treatment with SCH 39166. Results indicate a significant, dose-dependent up-regulation of D-1 receptor number in both caudate and putamen, with no changes in either D-2 receptors in the striatal regions or $5HT_2$ receptors in the frontal cortex. These data, therefore, indicate that SCH 39166 is a selective antagonist at D-1 receptors in the CNS of nonhuman primates.

D-1 dopamine receptors SCH 39166 Up-regulation Rhesus monkeys

CHRONIC administration of both agonists and antagonists of neurotransmitters has been shown to produce changes in receptor densities. Agonists generally produce decreases, or down-regulation, of the receptor number following chronic administration. Conversely, antagonists produce up-regulation, or an increase in receptor density, with repeated dosing. The changes seen in receptor number are specific only to the receptor(s) to which the drug binds, and usually are not accompanied by changes in the affinity of the receptor(s). The phenomena of up- and down-regulation of receptors have been demonstrated using compounds selective for a number of different neurotransmitters, including those agents which bind to dopaminergic receptors.

Five distinct dopamine receptors have been identified by their binding and second messenger properties (6) or by cloning techniques (11–13). Compounds which are selective for the D-2 receptor subtype, such as haloperidol, have been shown to produce up-regulation of D-2 receptors, and not D-1 receptors, in rats. Selective up-regulation of D-1 receptors was likewise demonstrated in rats following chronic administration of the D-1 selective antagonist, SCH 23390 (4). At the current time, there are no data on drug-induced changes in the density of D-3, D-4 or D-5 receptors.

More recently, a new D-1 selective antagonist, SCH 39166 [(−)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5-H-benzo[d]naphtho-{2,1b}azepine], has been characterized (3). In vitro, SCH 39166 was demonstrated to be 270 and 88 times more potent at D-1 receptors than at D-2 or $5HT_2$ sites, respectively (3). In vivo, SCH 39166 was shown to bind selectively to D-1 receptors in rat striatum, and selectively protected D-1 receptors from inactivation by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (7). Behaviorally, SCH 39166 exhibited a preclinical profile similar to other antipsychotics and has been shown to possess a longer duration of action in primates than its predecessor, SCH 23390 (3). The promising preclinical data have resulted in initiation of clinical trials of SCH 39166 for the treatment of schizophrenia in humans (2).

Despite the wealth of studies describing the biochemical and behavioral effects of SCH 39166, there are no data in primates which biochemically confirm the D-1 mechanism of the drug. Therefore, the present studies were designed to examine the ability SCH 39166 to produce a selective up-regulation of D-1 dopamine receptors in rhesus monkeys. The rhesus monkey brain tissue used in these studies became available as the result of preclinical toxicology studies required by the FDA prior to the start of clinical trials. Selective up-regulation of D-1 receptors by SCH 39166 would provide the first biochemical confir-

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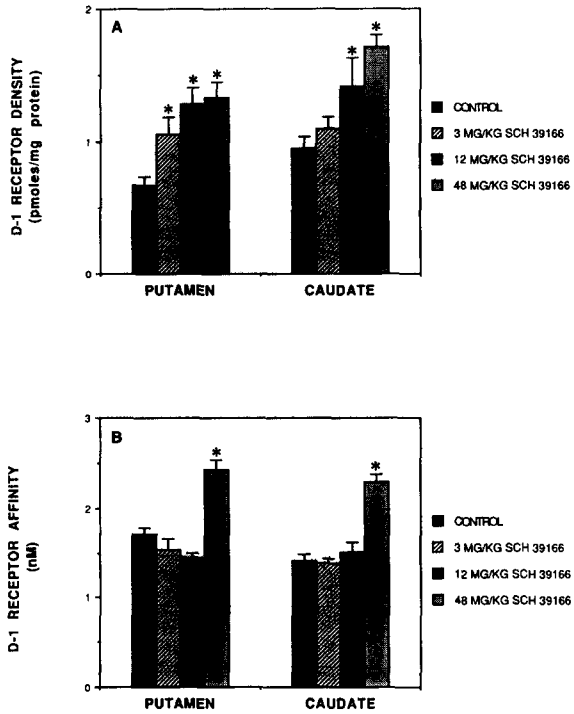

FIG. 1. D-1 receptor density (A) and affinity (B) determined from saturation analyses using ^{3}H-SCH 23390 in caudate and putamen of rhesus monkeys chronically treated with SCH 39166. Each group was composed of samples from 6 individual animals, 3 male and 3 female. Significant increases in receptor B_{max} or K_D, when compared to vehicle treatment, are indicated by an *.

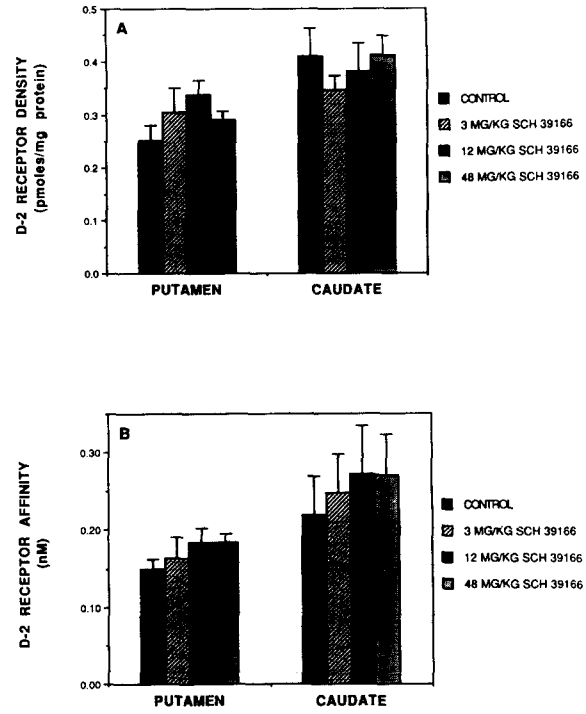

FIG. 2. D-2 receptor density (A) and affinity (B) measured in caudate and putamen of the the same animals as in Fig. 1 using ^{3}H-spiperone. No significant changes in either density or affinity were determined.

mation of its in vivo D-1 selectivity and CNS penetrability in nonhuman primates.

METHOD

Twenty-four male and female rhesus monkeys were separated into four groups (3 male and 3 female per group) and treated orally with either vehicle or SCH 39166 (3, 12 or 48 mg/kg) daily for three months. Twenty-four hours following the last treatment, all animals were sacrificed and caudate, putamen and frontal cortex tissues were dissected and frozen at −80°C until processed into membranes.

Membranes from the three rhesus monkey brain regions were prepared by homogenizing the tissue in 40 volumes (w/v) of 50 mM Tris-HCl, pH 7.4 (Buffer A), using a Brinkman Polytron. Following centrifugation at 20,000 × g for 10 min, the supernatant was discarded and the pellet was resuspended in Buffer A and centrifuged again at the same speed. The supernatant was again discarded and the resulting pellet was resuspended in Buffer A containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. The final protein concentrations of the membranes from each group were determined using a bicinchoninic (BCA) assay kit (Pierce). Membrane aliquots were frozen at −80°C until used in subsequent binding studies.

Saturation analyses of binding to D-1, D-2 and $5HT_2$ receptors were performed using ^{3}H-SCH 23390, ^{3}H-spiperone, and ^{3}H-ketanserin, respectively (1,8). Aliquots of both caudate and putamen membranes were incubated with increasing concentrations of ^{3}H-SCH 23390 and ^{3}H-spiperone, while frontal cortex membranes were incubated with ^{3}H-ketanserin, for 20 min at 37°C. Nonspecific binding at each concentration of ligand was determined in the presence of either 1 μM SCH 23390, 10 μM butaclamol or 10 μM methysergide for the D-1, D-2, and $5HT_2$ receptors, respectively. Following incubation, the reactions were terminated by filtration over GF/B glass fiber filters. The filters were subsequently washed with cold Buffer A using a Skatron filtration apparatus. Ready-Safe scintillant was added to the filters and the vials were incubated overnight. A Beckman liquid scintillation counter (50% efficiency) was used to determine the amount of bound radioligand. The data from each group were then analyzed according to the method of Scatchard (9), and the values for receptor affinity (K_D) and receptor density (B_{max}) for each treatment were determined using linear regression analysis.

RESULTS

Membranes prepared from the caudate and putamen of rhesus monkeys which had been chronically treated with SCH 39166 (3, 12 and 48 mg/kg, PO) exhibited a significant, dose-dependent increase in D-1 receptor B_{max} in both the caudate and putamen (Fig. 1). In the putamen, the increase in B_{max} was statistically significant ($p<0.05$, Duncan's multiple range test) at all three doses of SCH 39166 tested, while in the caudate, only the 12 and 48 mg/kg doses produced a significant up-regulation. Analysis of the affinity constants of D-1 receptors in the caudate and putamen of rhesus monkeys (Fig. 1) indicated a significant increase in K_D in both regions at only the 48 mg/kg dose of SCH 39166 ($p<0.05$, Duncan's multiple range test). When the plasma levels of both SCH 39166 and its major metabolite were determined, measurable amounts of the drug-derived material were present 24 hours after the last administration only in those animals which had received the 48 mg/kg dose (unpublished data). The increase in K_D at the 48 mg/kg dose, therefore, may be the

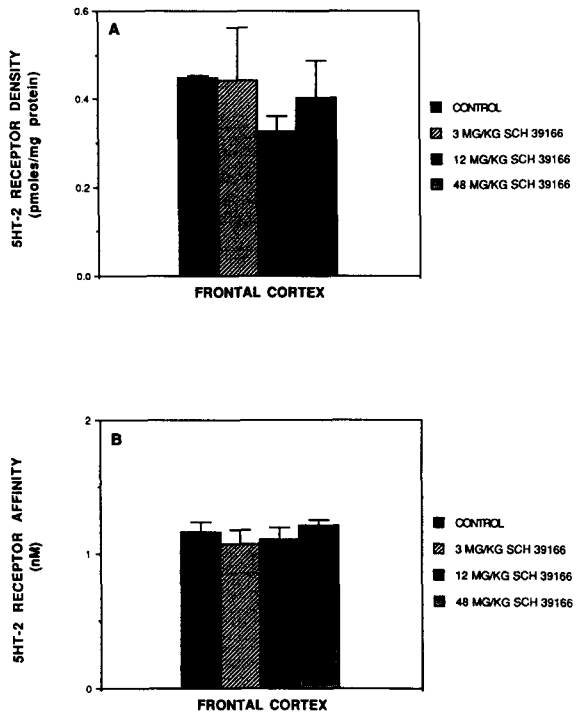

FIG. 3. B_{max} and K_D values obtained from saturation studies using ^{3}H-ketanserin in frontal cortex of rhesus monkeys. Each group was composed of 3 animals. No significant differences between treatment groups were noted.

result of residual drug still bound to the D-1 receptors.

An examination of the caudate and putamen from rhesus monkeys for changes in D-2 receptor number and affinity (Fig. 2) revealed no significant differences between treatment groups. Likewise, there was no significant change in either the density or affinity of $5HT_2$ receptors in the frontal cortex of rhesus monkeys at any of the doses of SCH 39166 tested (Fig. 3). These data indicate that chronic treatment with SCH 39166 did not result in changes in the binding characteristics of these receptor subtypes.

DISCUSSION

Three specific conclusions can be drawn from this work: first, SCH 39166 is able to cross the blood-brain barrier in primates. Although this observation is obvious, it is the first biochemical data to indicate that the drug does enter the CNS of monkeys and is not metabolized to a nonpenetrable analogue. This conclusion was confirmed recently by Sedvall and coworkers (10), who used positron emission tomography to localize radioactive SCH 39166 to the striatum and neocortex of cynomolgus monkeys.

The second conclusion is that SCH 39166 binds selectively to D-1 receptors in the striatum of rhesus monkeys. This conclusion is based on the selectivity of the up-regulation of D-1 receptors. While the lack of an effect at D-2 receptors was not unexpected, the inability of SCH 39166 to affect $5HT_2$ receptor density suggests that SCH 39166, unlike SCH 23390, does not bind to $5HT_2$ receptors, in vivo. These data are similar to findings in the rat, where SCH 39166 was demonstrated to be more selective for D-1 receptors, as opposed to $5HT_2$ receptors, in vivo, than was its analogue, SCH 23390 (7). Likewise, autoradiographic studies using rat brains demonstrated that ^{3}H-SCH 39166 labeled lamina IV of the cortex and the choroid plexus less densely than did ^{3}H-SCH 23390, again indicating the poor affinity of SCH 39166 for 5-HT_2 receptors (14).

Finally, these studies demonstrate that SCH 39166 is functioning as an antagonist at the D-1 site in monkeys. As mentioned above, neurotransmitter agonists produce down-regulation of receptors following repeated administrations, while antagonists increase the number of binding sites.

The finding that SCH 39166 binds selectively in vivo to D-1 dopamine sites has important implications for its potential clinical use as an antipsychotic. The currently available antipsychotics are either nonselective dopamine antagonists or are D-2 selective. One of the side effects of acute treatment associated with these antipsychotics is the development of a movement disorder, referred to as extrapyramidal syndrome. Studies in cebus monkeys have demonstrated that these movement disorders are produced by repeated administration of the D-2 antagonist, haloperidol, but are not seen following repeated treatment with SCH 39166 (5). The studies presented herein confirm that SCH 39166 is D-1 selective in nonhuman primates and suggest that this D-1 selectivity is the mechanism responsible for its improved side-effect profile.

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