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# Low affinity binding of the classical $D_1$ antagonist SCH23390 in rodent brain: Potential interaction with $A_{2A}$ and $D_2$-like receptors

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ABSTRACT

Whereas structurally dissimilar $D_1$ antagonists competing for [$^3$H]-SCH23390 binding recognize primarily one site in striatum, two distinct affinity states are observed in both amygdala and hippocampus. The binding profile of SCH23390 is similar in both of these regions, with the high affinity site ($K_D$ ~0.4 nM) consistent with $D_1/D_5$ receptors. The appearance of the low affinity site ($K_D$ ~300 nM) is dependent upon the absence of $MgCl_2$, but independent of $D_1$ expression (i.e., still present in $D_1$ knockout mice). Although the density of high affinity state receptor is lower in hippocampus or amygdala of $D_1$ knockout mice, some residual binding remains, consistent with the known expression of $D_5$ receptors in these regions. Remarkably, in hippocampus, the affinity of the low affinity site is shifted rightward in the presence of the $D_2$ antagonist domperidone and is largely absent in the hippocampus of $D_2$ knockout animals. Additionally, this site is also shifted rightward in the presence of the $A_{2A}$ ligands SCH58261, CSC, or NECA, or in the absence of $A_{2A}$ receptors. The affinity of SCH23390 for this low affinity site is greater than seen for SCH23390 binding to $D_2$ receptors in heterologous expression systems, consistent with the hypothesis that both $D_2$ and $A_{2A}$ receptors are involved in the low affinity binding site. Therefore, we suggest that the heteromerization of $D_2$ and $A_{2A}$ receptors reported previously in vitro also may occur in the brain of both rats and mice.



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*Abbreviations:*
AC, adenylate cyclase
cAMP, cyclic AMP, adenosine 3′,5′-cyclic monophosphate
CGS21680, 2-[4-(2-carboxyethyl)-phenylethylamino]-5′*N*-ethyl-carboxamido-adenosine
CHO, Chinese Hamster Ovary cell-line
CSC, 8-(3-chlorostyryl)caffeine
GPCR, G protein-coupled receptor
HEK, Human Embryonic Kidney cell-line
HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
$IC_{50}$, concentration inhibiting 50% of total binding
$K_{0.5}$, concentration corrected $IC_{50}$ (apparent affinity constant) when $n_H \neq 1.0$
$K_H$, affinity constant for high affinity state
$K_I$, affinity constant ($n_H = 1.0$)
$K_L$, affinity constant for low affinity state
NECA, 5′-*N*-ethylcarboxamide-adenosine
$n_H$, Hill coefficient
$R_H$, relative amount (percentage) of receptor in the high affinity state
SCH23390, 7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine
SCH58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3e]-1,2,4-triazolo[1,5c]pyrimidine

## 1. Introduction

Dopamine receptors comprise a subfamily of G protein-coupled receptors (GPCRs) encoded by five distinct genes ($D_1$, $D_2$, $D_3$, $D_4$, $D_5$). Functionally, $D_1$-like receptors ($D_1$ and $D_5$) are characterized by their ability to stimulate adenylate cyclase (AC) (Garau et al., 1978; Kebabian and Calne, 1979). Radioreceptor binding studies, autoradiographic, immunohistochemical, and *in situ* data clearly show that $D_1$ receptors are present in the amygdala (Dawson et al., 1988; Huang et al., 1992; Hurd et al., 2001; Mansour et al., 1992; Savasta et al., 1986; Sunahara et al., 1990), yet $D_1$-like receptors in the amygdala do not couple to activation of AC (Kilts et al., 1988; Leonard et al., 2003a,b; Mailman et al., 1986). Thus, the mechanisms by which $D_1$ receptors signal in this region remain unknown.

The current work was sparked by the surprising observation that, in the amygdala, but not in the striatum, the $D_1/D_5$ antagonist SCH23390 recognizes two clearly different affinity states (Leonard et al., 2003b). SCH23390 has proven very useful in ascribing functions and/or behaviors to $D_1$-like receptor activation due to its >500 fold $D_1$:$D_2$ selectivity and low affinity for most other neuroreceptors (see http://pdsp.cwru.edu/pdsp.asp). SCH23390 cannot, however, distinguish between $D_1$ and $D_5$ receptors. In the current work, we compare SCH23390 binding in the amygdala, striatum, and hippocampus to determine the nature of this unexpected low affinity SCH23390 binding site.

Of these three regions, the density of $D_1$ receptors is highest in the striatum, followed by the amygdala, and then hippocampus, whereas the hippocampus contains the highest density of $D_5$ receptors (Boyson et al., 1986; Montague et al., 2001). $D_1$-like receptors are believed to perform diverse physiological roles in these regions. For example, in the striatum, $D_1$-like receptors play a role in posture and the initiation of movement (Wang et al., 1998), whereas in the amygdala they modulate drug-reward and fear responses (Callahan et al., 1995; Greba and Kokkinidis, 2000). Hippocampal $D_1$-like receptors participate in learning and memory, likely through modulation of cAMP synthesis (Matthies et al., 1997; Otmakhova and Lisman, 1996).

Recent work has shown that many GPCRs, including the dopamine receptors, may evoke physiological responses through interactions with other GPCRs. $D_1$ receptors have been shown to interact with $A_1$ adenosine and NMDA