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Short communication

# Dopamine autoreceptor reserve in vitro: possible role of dopamine $D_3$ receptors

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**Abstract**

Receptor alkylation in vivo with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) decreased the potency, but not the efficacy, of the dopamine $D_3$ receptor-preferring agonist 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) at synthesis modulating dopamine autoreceptors in striatal and olfactory tubercle slices stimulated by 30 mM $K^+$. In contrast, 7-OH-DPAT was ineffective in slices exposed to forskolin (10 $\mu$M). The results support the presence of autoreceptor reserve in vitro, and the partial involvement of dopamine $D_3$ receptors in the autoregulation of dopamine synthesis.

*Keywords:* Receptor reserve; Dopamine $D_3$ receptor; 7-OH-DPAT (7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin)

## 1. Introduction

Pretreatment in vivo with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) markedly inhibits binding in vitro of most dopamine receptor ligands to dopamine receptors. However, a recent study by Levant (1995) found that binding in vitro of the dopamine $D_3$ receptor-preferring ligand 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) was unaffected by EEDQ pretreatment in vivo unless animals were depleted of synaptic dopamine prior to EEDQ treatment. Since dopamine binding sites can be protected from EEDQ blockade by dopamine itself (Hamblin and Creese, 1983), it is possible that the high affinity of $D_3$ receptors for dopamine selectively protects this subtype from EEDQ alkylation. Thus, EEDQ may be an important tool to discern dopamine $D_3$ vs. $D_2$ receptor function. Since dopamine $D_3$ receptors appear to be involved in the autoregulation of dopamine synthesis (Aretha et al., 1995; Gilbert and Cooper, 1995; Gobert et al., 1995; Meller et al., 1993), the present study investigated the functional consequences of EEDQ pretreatment in vivo on agonist effects of 7-OH-DPAT at synthesis modulating dopamine autoreceptors in vitro in slices from either rat striatum or olfactory tubercles.

## 2. Materials and methods

The inhibition of either $K^+$- (30 mM) or forskolin- (10 $\mu$M) stimulated 3,4-dihydroxyphenylalanine (DOPA) accumulation (in the presence of *m*-hydroxybenzylhydrazine) was used as an index of autoreceptor activity (Clark et al., 1991). 24 h following treatment with either vehicle (50% ethanol) or EEDQ (6 mg/kg s.c.), male Sprague-Dawley rats were decapitated and either striata or olfactory tubercles dissected and cross-chopped (0.3 $mm^2$). Striatal or olfactory tubercle slices were pooled and placed in oxygenated (95% $O_2$, 5% $CO_2$) Krebs-Ringer-MOPS buffer consisting of 128 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11.1 mM glucose, 100 $\mu$M *l*-tyrosine, and 15.8 mM 3-[*N*-morpholino]propanesulphonic acid (MOPS). After oxygenation, the buffer pH was adjusted to 7.4 with NaOH and maintained at 37°C. After a 5 min preincubation period, slices were washed and resuspended in fresh buffer. Subsequently, 0.5 ml aliquots of slices were delivered to incubation tubes and equilibrated in a shaking water bath. Agonists, when present, were added 5 min prior to stimulation by either $K^+$ (30 mM final) or forskolin (10 $\mu$M final). 10 min follow-

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ing stimulation, the aromatic decarboxylase inhibitor *m*-hydroxybenzylhydrazine (NSD-1015, 200 μM) was added to allow for the accumulation of DOPA. Samples were oxygenated, tubes capped, and incubated in a shaking water bath for an additional 30 min. Previous studies have confirmed the linearity of DOPA accumulation over this time period (Wolf et al., 1986). Reactions were terminated by acidification with $HClO_4$ (0.1 N final), and α-methyl DOPA was used as an internal standard. Slices were sonically disrupted and centrifuged for 5 min at 10 000 × *g*. To concentrate catechols, 0.8 ml supernatant from each tube was added to 0.1 ml Tris base (2.55 M), mixed, and

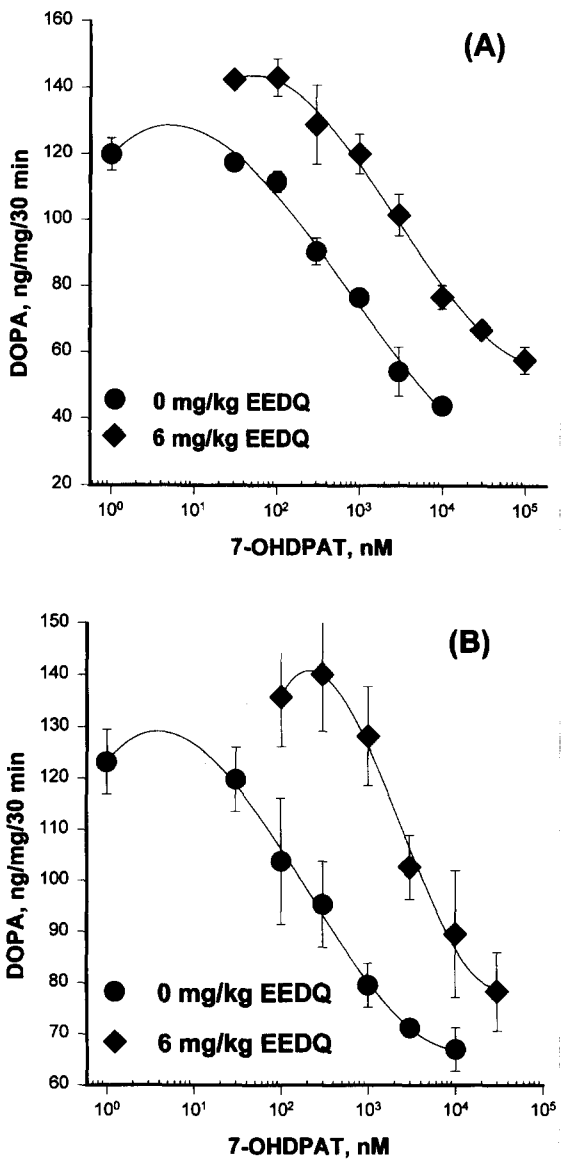


Fig. 1. Effect of receptor alkylation on the agonist effects of 7-OH-DPAT at synthesis modulating dopamine autoreceptors in striatal (A) and olfactory tubercle (B) slices in vitro: dose-response curves reflecting inhibition of $K^+$-stimulated DOPA accumulation by 7-OH-DPAT in striatal slices from either vehicle pretreated (circles), or EEDQ pretreated (diamonds) rats are represented using a third-order regression. Each point represents mean ± S.E.M. from 3–4 separate determinations performed in triplicate.

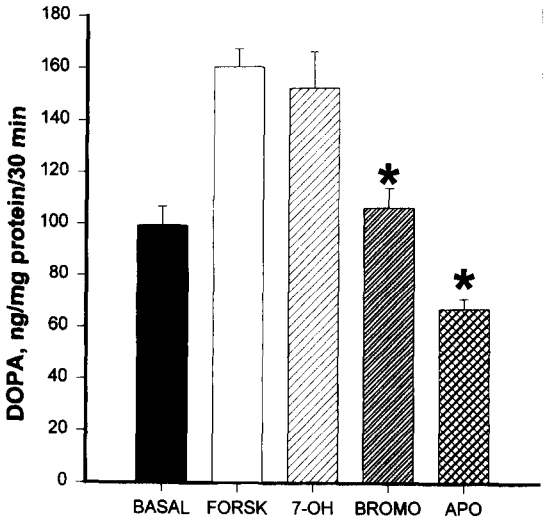


Fig. 2. Differential coupling of dopamine autoreceptor stimulation to inhibition of tyrosine hydroxylase activity: the ability of 1 μM 7-OH-DPAT, bromocriptine and apomorphine to inhibit forskolin-stimulated dopamine synthesis was tested in striatal slices in vitro. Each bar represents mean ± S.E.M. of 4–8 separate determinations. Data are represented as DOPA, ng/mg protein/30 min. * $P < 0.01$ vs. forskolin-stimulated samples, one-way ANOVA with post-hoc Newman-Keuls.

poured through microcolumns containing activated alumina (Bioanalytical Systems, West Lafayette, IN). Columns were then subjected to centrifugation, washed twice with 0.25 ml $H_2O$, and catechols subsequently eluted with 0.15 ml oxalic acid (100 mM). 15 μl of alumina-extracted sample was injected onto a high performance liquid chromatography (HPLC) system consisting of a Scientific Systems pump (State College, PA), a 5 μm C-8 reverse-phase column (Bioanalytical Systems), and a LC3 electrochemical detector with dual glassy carbon electrode maintained at 0.7 V vs. Ag/AgCl reference electrode (Bioanalytical Systems). Catechol content of samples was based on internal and external standards. Separation was afforded by a mobile phase consisting of: 0.8 mM octyl sulfonic acid, 0.1 mM EDTA and 5% methanol in a 0.1 M monobasic sodium phosphate buffer adjusted to a pH of 2.8 with phosphoric acid. The mobile phase was pumped at a flow rate of 0.6 ml/min.

The $ED_{50}$ values and maximal effects were determined using Marquette's least squares non-linear regression (Graphpad, ISI Software, Philadelphia, PA), and are reported as the mean ± S.E.M. of 3–4 separate determinations performed in triplicate. Statistical analyses included one-way analysis of variance (ANOVA), and Student's *t*-test as applicable.

## 3. Results

7-OH-DPAT stimulated dopamine autoreceptors in both striatal and olfactory tubercle slices in vitro, decreasing $K^+$-stimulated DOPA accumulation maximally to 30.2 ±

5.5 and 64.5 ± 3.2 ng DOPA/mg protein/30 min, respectively (Fig. 1). 7-OH-DPAT displayed greater potency at autoreceptors on mesolimbic dopamine terminals ($ED_{50}$ = 348 ± 102 nM) than nigrostriatal terminals (949 ± 190 nM, $P < 0.05$). EEDQ pretreatment decreased the potency of 7-OH-DPAT in both striatal slices ($ED_{50}$ = 4787 ± 140 nM) and olfactory tubercle slices ($ED_{50}$ = 2550 ± 113 nM), with no significant difference in maximal efficacy ($P > 0.05$).

To determine the potency of 7-OH-DPAT at dopamine autoreceptors coupled to adenylyl cyclase, the ability of 7-OH-DPAT to inhibit forskolin-stimulated dopamine synthesis was tested. Incubation with 1 μM 7-OH-DPAT failed to affect forskolin-stimulated dopamine synthesis in striatal slices (Fig. 2), in contrast to its effects under hyperkalemic conditions (Fig. 1). Furthermore, a dose response to 7-OH-DPAT (0.01–10 μM) revealed no significant effect on forskolin-stimulated dopamine synthesis ($F(1,5) = 1.218$, $P > 0.05$) in striatal slices (data not shown). However, the nonselective dopamine agonists, bromocriptine (1 μM) and apomorphine (1 μM) did significantly inhibit forskolin-stimulated dopamine synthesis ($P < 0.01$) in striatal slices, at a dose that was ineffective for 7-OH-DPAT (Fig. 2).

## 4. Discussion

The present study demonstrated the ability of the dopamine $D_3$ receptor-preferring dopamine receptor ligand, 7-OH-DPAT, to differentially stimulate synthesis modulating dopamine autoreceptors in vitro. The greater potency of 7-OH-DPAT on limbic vs. striatal nerve terminals is consistent with previous findings in vivo (Aretha et al., 1995) and with the greater density of 7-OH-DPAT binding sites in olfactory tubercles (Landwehrmeyer et al., 1993; Levèsque et al., 1992). Additionally, pretreatment in vivo with EEDQ decreased the potency of 7-OH-DPAT in both striatal and olfactory tubercle slices; however, the maximal efficacy was unchanged in either region. This rightward shift in potency, and unaltered maximal efficacy following EEDQ pretreatment is consistent with the presence of autoreceptor reserve (cf. Meller et al., 1986). To our knowledge, this is the first demonstration of receptor reserve for synthesis modulating dopamine autoreceptors in striatal and olfactory tubercle slices in vitro.

Previous studies, using the inhibition of forskolin-stimulated dopamine synthesis as an index of autoreceptor activity, failed to demonstrate the presence of reserve for synthesis modulating dopamine autoreceptors in vitro (Bohmaker et al., 1989). Since there is little evidence to suggest that dopamine $D_3$ receptors are coupled to the inhibition of adenylyl cyclase (Van Leeuwen et al., 1995), it is possible that the use of forskolin-stimulated dopamine synthesis as an index of autoreceptor activity could mask any contribution of dopamine $D_3$ receptors to the autoregulation of dopamine synthesis. This possibility is further supported by the inability of 7-OH-DPAT to inhibit forskolin-stimulated dopamine synthesis while compounds that have greater potency at dopamine $D_2$ receptors completely block the effect of forskolin. The differential ability of 7-OH-DPAT to inhibit $K^+$- vs. forskolin-stimulated dopamine synthesis might reflect the presence of a functional subtype of dopamine autoreceptor coupled to a second messenger other than adenylyl cyclase. We have shown previously that dopamine autoreceptor stimulation decreases the phosphorylation state of striatal tyrosine hydroxylase (Salah et al., 1989). The possible involvement of multiple coupling pathways mediating autoreceptor control of tyrosine hydroxylase activity may lend functional significance to the multiple phosphorylation sites (e.g. $Ser^{19}$, $Ser^{31}$ and $Ser^{40}$) on tyrosine hydroxylase (Haycock and Haycock, 1991).

While EEDQ pretreatment in vivo did not diminish binding of [$^3$H]7-OH-DPAT in vitro (Levant, 1995), the present study demonstrates a decreased functional potency of 7-OH-DPAT in vitro. If dopamine $D_3$ receptors are selectively spared from EEDQ alkylation, and if dopamine $D_3$ receptors were solely responsible for the autoregulation of dopamine synthesis, the EEDQ pretreatment would not be expected to alter the functional potency of 7-OH-DPAT. The data in Fig. 1 suggest that autoregulation of dopamine synthesis is not mediated solely by dopamine $D_3$ receptors. Although 7-OH-DPAT displays a certain binding preference for dopamine $D_3$ receptors vs. $D_2$ receptors (Levant et al., 1995; Levèsque et al., 1992), the degree of selectivity depends on both the in vitro assay conditions and the affinity state of the receptors (Burris et al., 1995; Gonzalez and Sibley, 1995). Since the functional selectivity of 7-OH-DPAT has yet to be established, a contribution of dopamine $D_2$ receptors to the autoregulation of dopamine synthesis observed in the present study is likely. However, Meller et al. (1993) reported that following pretreatment with EEDQ, the potency of several agonists in the γ-butyro-lactone model in vivo correlated with their binding affinities at dopamine $D_3$ but not $D_2$ receptors. A selective blockade of dopamine $D_2$ receptors (i.e. relative to $D_3$ receptors) by EEDQ may account for this correlation by excluding any contribution of dopamine $D_2$ receptors to the inhibition of dopamine synthesis.

Prima faci, the present data confirm the presence of dopamine autoreceptor reserve in vitro. The involvement of dopamine $D_3$, as well as $D_2$ receptors in the autoregulation of dopamine synthesis is probable. Given the differential potency of 7-OH-DPAT at inhibiting $K^+$- vs. forskolin-stimulated dopamine synthesis, dopamine autoreceptors may also be coupled to various second messengers. These findings, combined with evidence for differential blockade of dopamine $D_3$ vs. $D_2$ receptor binding sites by EEDQ pretreatment, may indicate a possible role of dopamine $D_3$ receptors in the phenomenon of autoreceptor reserve.

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