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COMPARISON OF THE EFFECTS OF RECENTLY DEVELOPED α_2-ADRENERGIC ANTAGONISTS WITH YOHIMBINE AND RAUWOLSCINE ON MONOAMINE SYNTHESIS IN RAT BRAIN

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Abstract—The effects of two recently developed α_2-adrenergic antagonists, RX 781094 and WY 26703, on the synthesis of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in rat brain were compared to those of yohimbine, its diastereoisomer rauwolscine, and mianserin. Intraperitoneal administration of these compounds increased cortical NE synthesis with the potency order: yohimbine, RX 781094, WY 26703 > rauwolscine > mianserin. Within a similar dose range, yohimbine, rauwolscine and WY 26703 also stimulated striatal DA synthesis and decreased hypothalamic 5-HT synthesis, while RX 781094 and mianserin were very weak or inactive. Yohimbine and the structurally-related WY 26703 were also active as DA antagonists in the γ-butyrolactone model for DA autoreceptor function. Based on the drug-induced changes in monoamine synthesis as indication of receptor-mediated events, RX 781094 has greater selectivity as an α_2-antagonist than compounds structurally related to yohimbine.

Yohimbine has long been regarded and utilized as a standard α_2-adrenergic (α_2) antagonist (see Ref. 1 for review). Its diastereoisomer, rauwolscine (α-yohimbine), exhibits potent α_2-antagonism as well and has recently been tritiated for use in an *in vitro* binding assay to identify α_2 binding sites [2]. In addition to earlier claims that yohimbine exhibits serotonin-like properties [3], there is also neurochemical evidence that yohimbine is a dopamine antagonist [3–5]. It is because of the relative non-specificity of yohimbine that there have been efforts to develop more potent selective α_2-antagonists.

In this report we compare the effects of yohimbine with its isomer rauwolscine and two recently described α_2-antagonists, WY 26703 [6] and RX 781094 [7, 8] (see Fig. 1) on rat brain monoamine [norepinephrine (NE), dopamine (DA), serotonin (5-HT)] synthesis.

MATERIALS AND METHODS

Animals. Male, Sprague–Dawley rats (125–175 g; Charles River Breeding Laboratories, Wilmington, MA) were used in all experiments. Animals were housed under a 12-hr light:12-hr dark cycle having free access to laboratory rat chow and tap water.

In vivo *monoamine synthesis.* The rates of NE-, DA- and 5-HT synthesis in rat brain were estimated by measuring the formation of neocortical (cingulo-parietal area) [9] dihydroxyphenylalanine (DOPA), striatal DOPA and hypothalamic 5-hydroxytryptophan (5-HTP), respectively, after inhibition of aromatic amino acid decarboxylase by *m*-hydroxybenzylhydrazine dihydrochloride (NSD 1015) [10]. In pilot studies, it was determined that a 125 mg/kg (i.p.) dose of NSD 1015 was required for maximal formation of DOPA and 5-HTP at 30 min in all three brain regions studied. DOPA and 5-HTP were assayed by the methods of Hefti *et al.* [11] and Reinhard *et al.* [12] using high performance liquid chromatography with electrochemical detection.

γ-Butyrolactone (GBL) procedure. Compounds were tested for antagonist activity at the DA autoreceptor *in vivo* using the GBL model of Walters and Roth [13]. Briefly, animals were treated with the test drug (i.p.) 10 min before they received the potent DA agonist *n*-propylnorapomorphine (NPNA; 0.06 μmole/kg, i.p.). Five minutes later, GBL (750 mg/kg, i.p.) was administered followed after another 5 min with NSD 1015 (100 mg/kg, i.p.).

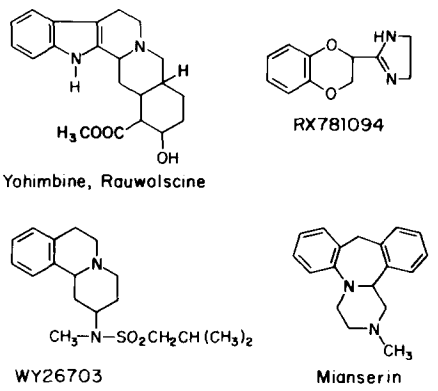

Fig. 1. Chemical structures of α_2-adrenergic antagonists.

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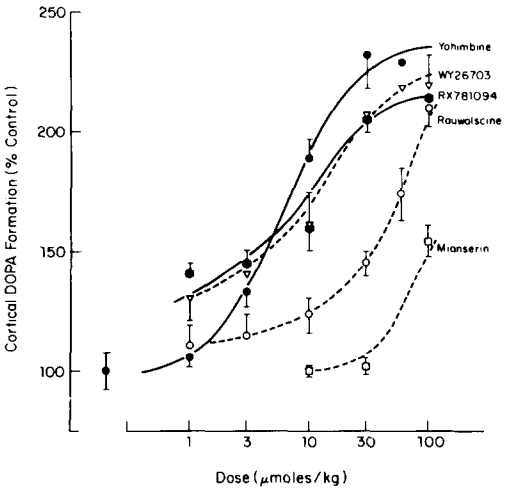

Fig. 2. Effects of α_2-adrenergic antagonists on cerebral cortical (cingulo-parietal) NE synthesis. Rats were administered the various compounds or vehicle (0.25% methylcellulose) at the indicated doses (i.p.). Twenty minutes later they received NDS 1015 (125 mg/kg, i.p.) and were killed after 30 min. The 20-min pretreatment time was determined to give the peak effect of all of the α_2-antagonists on cortical DOPA synthesis (data not shown). Values are percent of control (means ± S.E.M.) DOPA formation for two separate experiments (N = 10/dose). Overall mean control value (± S.E.M.) is 0.52 ± 0.03 pmole/mg tissue (N = 45).

Thirty minutes later, the rats were killed by decapitation. Blockade of the reduction in the GBL-induced rise in striatal levels of DOPA by NPNA was taken as an indication of DA autoreceptor antagonism. The dose of NSD 1015 (100 mg/kg, i.p.) used here produced maximal elevations of DOPA in the striatum; the higher dose used in the regional studies (see above) was not required. Similar levels of striatal DOPA with or without GBL co-administration have been reported using RO 44602 (800 mg/kg, i.p.) as the decarboxylase inhibitor [13].

Drugs. The following compounds were obtained commercially: NSD 1015 (Aldrich Chemical Co., Milwaukee, WI), GBL (Sigma Chemical Co., St. Louis, MO), yohimbine (Regis Chemical Co., Morton Grove, IL), and rauwolscine (Carl Roth, Karlsruhe, West Germany). WY 26703 (Wyeth Laboratories, Philadelphia, PA) and mianserin (Organon Inc., West Orange, NJ) were received as gifts. NPNA and RX 781094 [2-(2,3-dihydro-1,4-benzodioxan-2-yl)-4,5-dihydro-1*H*-imidazole HCl] were synthesized in the Medicinal Chemistry Department at the Merck Sharp & Dohme Research Laboratories, West Point, PA.

RESULTS

In vivo *monoamine synthesis.* After intraperitoneal administration, all five α_2-antagonists increased cortical DOPA accumulation (NE synthesis) dose dependently (Fig. 2). Yohimbine, RX 781094 and WY 26703 exhibited similar high potencies while mianserin, a 5-HT antagonist [14] with α_2-antagonist properties [15], was quite weak in this regard (Table 1). Interestingly, rauwolscine showed about one-sixth the potency of yohimbine (Fig. 2, Table 1) in enhancing cortical DOPA formation.

The effects of these α_2-antagonists on brain DA turnover were estimated by measuring DOPA formation in striatum (Fig. 3). Rauwolscine and yohimbine were equally potent in this regard, having roughly 2-fold higher potencies than WY 26703 (Table 1). These compounds, however, were considerably less potent than haloperidol, which increased striatal DOPA accumulation by 100% at about 0.3 μmole/kg (data not shown). Neither mianserin nor RX 781094 altered striatal DA synthesis at doses up to 100 μmoles/kg (Fig. 3).

With respect to 5-HT turnover, all of the α_2-antagonists except mianserin (data not shown) showed serotonin-like activity, reducing hypothalamic 5-HTP formation with the potency order: yohimbine ≥ rauwolscine > WY 26703 ≫ RX 781094 (Fig. 4, Table 1). For purposes of reference, two 5-HT agonists, lysergic acid diethylamide and lisuride, reduced 5-HTP accumulation by 25% at about 0.2

Table 1. Comparison of potencies of α_2-adrenergic antagonists on rat brain monoamine metabolism*

Compound	NE synthesis†	DA synthesis‡	5-HT synthesis§
Yohimbine	5.3 (4.0, 6.4)	7.3 (4.3, 11.3)	4.0 (2.7, 5.6)
RX 781094	6.6 (4.5, 9.0)	Inactive‖	136.1 (72.0, 745)
WY 26703	7.0 (2.8, 11.7)	14.7 (10.7, 20.1)	21.3 (14.2, 32.7)
Rauwolscine	33.8 (25.8, 44.7)	5.9 (3.8, 8.2)	7.7 (4.7, 13.8)
Mianserin	>100	Inactive‖	Inactive‖

* Values indicate the dose (μmoles/kg, i.p.) required to produce change in monoamine synthesis as a measure of *in vivo* potency. The potencies (±95% confidence limits) were calculated from combined data of two separate experiments (Figs. 1–3) using log probit analysis.

† Dose required to increase cortical DOPA formation to 160% control value.

‡ Dose required to increase striatal DOPA formation to 200% control value.

§ Dose required to decrease hypothalamic 5-HTP formation to 75% control value.

‖ Up to 100 μmoles/kg, i.p.

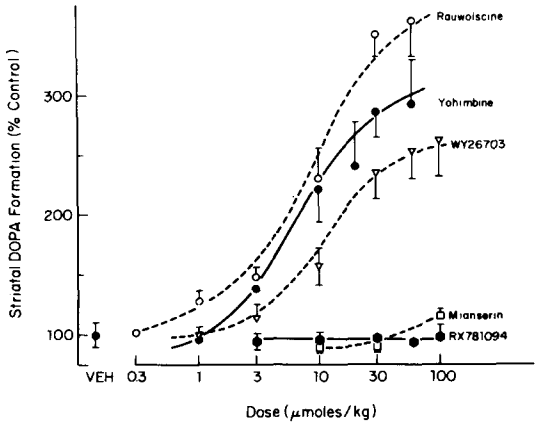

Fig. 3. Effects of α_2-adrenergic antagonists on striatal DA synthesis. Rats were treated as in Fig. 2. Values are percent of control (mean ± S.E.M.) DOPA formation for two separate experiments (N = 10/dose). Overall mean control value (± S.E.M.) is 8.5 ± 0.5 pmoles/mg tissue (N = 45).

and 0.5 μmole/kg (i.p.) respectively (data not shown).

GBL procedure. While at least ten times less potent than haloperidol, both yohimbine and WY 26703 were active in blocking the inhibition by NPNA of the GBL-induced rise in striatal DOPA formation (Table 2). At doses up to 100 μmoles/kg, neither mianserin nor RX 781094 was active in the GBL procedure (data not shown).

DISCUSSION

Over the last two decades, many studies have been reported which describe the effects of various

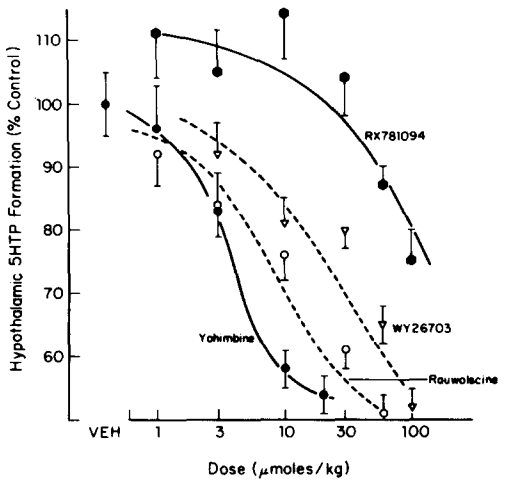

Fig. 4. Effects of α_2-adrenergic antagonists on hypothalamic 5-HT synthesis. Rats were treated as in Fig. 2. Values are percent of control (mean ± S.E.M.) DOPA formation for two separate experiments (N = 10/dose). Overall mean control values (± S.E.M.) is 1.64 ± 0.06 pmoles/mg tissue (N = 45).

Table 2. Effects of α_2-adrenergic antagonists in the GBL procedure

Treatment	Striatal DOPA accumulation* (pmoles/mg tissue)					
	Haloperidol		Yohimbine		WY 26703	
Controls (NSD 1015 alone)	7.0 ± 0.1		6.9 ± 0.3		7.5 ± 0.5	
GBL	21.8 ± 0.1		21.3 ± 1.7		25.6 ± 0.8	
		%		%		%
NPNA + GBL	13.8 ± 1.8	Inhibition†	14.9 ± 1.6	Inhibition	13.4 ± 0.8	Inhibition
Dose 1‡ + NPNA + GBL	(0.1)‡13.8 ± 2.1	0	(1) 11.7 ± 1.1	0	(3) 12.5 ± 0.9	0
Dose 2 + NPNA + GBL	(0.3) 14.4 ± 1.5	8	(3) 14.6 ± 1.8	0	(10) 16.1 ± 0.7	22
Dose 3 + NPNA + GBL	(1.0) 16.7 ± 2.1	36	(10) 14.9 ± 1.0	0	(30) 22.4 ± 2.6	73
Dose 4 + NPNA + GBL	(3.0) 23.9 ± 2.0	100	(30) 18.1 ± 1.6	50	(100) 24.9 ± 2.5	94

* Procedure as described in Materials and Methods. Values are $\bar{X}$ ± S.E.M. (N = 5).
† Percent inhibition of the effect of *n*-propylnorapomorphine (NPNA; 0.06 μmole/kg, i.p.) on GBL-induced rise in striatal DOPA formation.
‡ Dose of each compound (μmoles/kg, i.p.) appears in parentheses.

centrally-active agents on brain monoamine metabolism *in vivo*. From the results of these studies, a general picture has emerged which enables the tentative classification of some of the properties of compounds based on how they affect the turnover of brain NE, DA or 5-HT. Compounds such as clonidine and yohimbine, for example, decrease and increase cortical NE turnover by virtue of their abilities to stimulate or block α_2-receptors respectively [16–18]. Similarly, DA agonists (e.g. apomorphine) or antagonists (e.g. haloperidol) decrease or increase DA turnover in striatum [19–22], while serotonin agonists predictably reduce brain 5-HT turnover [23–25].

In the present studies, the rate of cortical DOPA accumulation within noradrenergic nerve terminals after decarboxylase inhibition was used as a specific index of α_2- (i.e. vs α_1) antagonist activity *in vivo*; the administration of the α_1-antagonists prazosin [26, 27] or corynanthine [28–30], another diastereomer of yohimbine, at doses up to 100 μmoles/kg, i.p., did not alter cortical DOPA formation (our unpublished observations). Another important consideration for the use of cortical DOPA formation for assessing α_2-antagonism is the possible contribution of DOPA formed within dopaminergic nerve terminals of the mesocortical pathway. It has been determined that the turnover of DA in the prefrontal cortex is 2- to 3-fold faster than that of NE [31]. The neocortical region chosen for our studies (cingulo-parietal; AP planes of Konig and Klippel [32], 2.0 to 6.0 mm), however, does not receive an appreciable DA input [33]. Furthermore, haloperidol, a potent and selective DA antagonist, did not affect cortical DOPA at doses (up to 3.8 mg/kg, i.p.) which elevated striatal DOPA formation by 3-fold (our unpublished observations).

It is evident from the present studies that the selected α_2-antagonists exhibited widely different selectivities with regard to *in vivo* monoamine turnover. Yohimbine was relatively potent in enhancing cortical and striatal DOPA formation and reducing hypothalamic 5-HTP accumulation, suggesting that this alkaloid exhibits α_2- and DA-receptor blocking properties as well as serotonin-like characteristics. This profile of activity for yohimbine is consistent with that obtained elsewhere [1, 3–5]. Interestingly, rauwolscine which differs from yohimbine only with respect to the orientation of the hydrogen and carboxymethyl groups on the asymmetric carbons at the 20- and 16-positions, respectively (Fig. 1), exhibited only about one-sixth the potency of yohimbine to increase cortical NE synthesis (Table 1). This reduced potency, however, would not be predicted from their binding affinities to α_2-receptors *in vitro*: in radioligand binding assays, rauwolscine actually shows a slightly higher affinity than yohimbine for these sites in brain [2, 34]. It is possible that rauwolscine enters the brain less readily than yohimbine after intraperitoneal administration, but this does not appear to be the case inasmuch as rauwolscine was equipotent with yohimbine on DA and 5-HT synthesis (Table 1, Figs. 3 and 4) in other brain regions taken from the same animal. Alternatively, differences in the affinities of these two alkaloids for α_1-binding may be involved. Yohimbine is about five times more potent than rauwolscine in competing for [^{3}H]prazosin or [^{3}H]WB 4101 binding in brain [28, 29], and it has been reported that prazosin, a specific α_1-antagonist, enhances the effect of yohimbine on cortical NE turnover [35]. This suggests that α_1-receptors modulate α_2-adrenergic control of cortical NE synthesis and may explain the higher potency of yohimbine *in vivo*.

With regard to the effects of the newer α_2-antagonists on monoamine turnover, RX 781094 displayed higher *in vivo* selectivity than WY 26703. While both RX 781094 and WY 26703 were essentially equipotent to yohimbine on cortical NE synthesis, RX 781094 had little, if any, influence on DA or 5-HT turnover (Table 1, Figs. 3 and 4). On the other hand, WY 26703, which bears structural resemblance to yohimbine and rauwolscine (Fig. 1), affected the synthesis of all three monoamines, although with weaker, but comparable, potencies than these alkaloids (Table 1).

It has been proposed that yohimbine enhances the turnover of striatal DA indirectly by increasing a noradrenergic–dopaminergic interaction; blockade of presynaptic α_2-adrenergic receptors enhances NE release resulting in postsynaptic α_1-adrenergic stimulation and increased DA turnover [22]. Supporting this possibility, the yohimbine-induced increase in DA turnover is blocked by prazosin [35]. On the other hand, there is biochemical evidence that yohimbine is a direct-acting DA antagonist [4, 5]. Consistent with this proposal are the findings that RX 781094 which is a potent α_2-antagonist does not alter striatal DA turnover either by measurement of DA metabolites [5, 36] or DA synthesis (Fig. 3, the present report). Furthermore, yohimbine and WY 26703 both acted as DA antagonists in the GBL model for autoreceptor function (Table 2), although they were less potent than haloperidol.

The reduction in hypothalamic 5-HT synthesis by the yohimbine-like α_2-antagonists may be due to 5-HT-mimetic properties, as mentioned earlier. An alternative explanation could involve an antagonism of noradrenergic-supported serotonergic neuronal firing and 5-HT synthesis of the raphe system which is mediated possibly through an α_1-adrenergic mechanism [37, 38]. This latter possibility does not appear to be involved because prazosin and corynanthine, which are much more potent α_1-antagonists than yohimbine [26–30], were less potent in reducing 5-HTP formation (ID_{25} 30–100 μmoles/kg; our unpublished observations).

Collectively, these findings suggest that newly developed α_2-antagonists such as WY 26703 which contain the benzoquinolizine structure of yohimbine will also interact with DA and 5-HT systems of the brain.

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