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α_1-Adrenergic and 5-HT_2-Serotonergic Effects of Some β-Alkoxy-β-phenylethylamines on Isolated Rat Thoracic Aorta

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ABSTRACT. 1. Ten racemic β-alkoxy-β-phenylethylamines were found to elicit concentration-dependent contraction of the isolated rat thoracic aorta with apparent pD_2 values in the 4.56–6.76 range. With one exception, which produces the same maximal contraction (E_{max}) as serotonin (5-HT), the E_{max} values attained with these compounds are lower than those produced by either 5-HT or norepinephrine (NE).

2. Pretreatment with either prazosin or ketanserin (10^{-8} M) leads in most cases to decreased E_{max} values and slopes in the dose–response curves. Apparent serotonergic (pD_2^S) and adrenergic (pD_2^A) pD_2 values going from 4.22 to 6.08 (pD_2^S) and from 3.87 to 5.27 (pD_2^A) were calculated from results obtained in the presence of prazosin or ketanserin, respectively.

3. In the 10^{-7}–10^{-5} M range, and in contrast with the results obtained with the previous compounds, BON [(±)-2-(2,5-dimethoxy-4-nitrophenyl)-2-methoxy-ethylamine] behaves as an antagonist to both 5-HT and NE (apparent pA_2=7.08 and 7.45, respectively) in this preparation. GEN PHARMAC 31;1:51–54, 1998. **© 1998 Elsevier Science Inc.**

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INTRODUCTION

Since the pioneering work of Alles (1932), structural variations on the catecholamine theme by the exchange or modification of substituents on the phenylethylamine ring and by the introduction of an α-methyl group to afford amphetamine analogues have yielded an enormous wealth of derivatives. Although many of these compounds bind fairly strongly to serotonin 5-HT_2 receptors (Glennon, 1994) and have been found to be psychotropic in human subjects (Shulgin and Shulgin, 1991), their basic pharmacology has not been extensively explored. An intriguing group of substances are the so-called BOX ("benzyloxy") series of β-alkoxy or -hydroxy analogues of several psychoactive ring-substituted phenylethylamines (the "2C" series) on which introduction of the β-oxygen atom was originally intended to mimic the physiological β-oxidation of dopamine to norepinephrine (Shulgin and Shulgin, 1991). The first members of this set to be described and evaluated in any way were the β-methoxy derivatives BOB [(±)-2-(4-bromo-2,5-dimethoxyphenyl)-2-methoxyethylamine], BOD [(±)-2-(2,5-dimethoxy-4-methylphenyl)-2-methoxyethylamine], BOH [(±)-2-(3,4-methylenedioxyphenyl)-2-methoxyethylamine] and BOM [(±)-2-(3,4,5-trimethoxyphenyl)-2-methoxyethylamine] (Lemaire *et al.*, 1985). BOB, BOD, BOH and BOM (which is the β-methoxy congener of mescaline) have been assessed in human volunteers and found to produce alterations of perception together with some unsettling indications of peripheral involvement, with the exception of BOM, which was inactive at the levels tested.

A number of ring-substituted phenylethylamines have been shown to contract the isolated rat thoracic aorta in a concentration-dependent way (Lobos *et al.*, 1992; Sáez *et al.*, 1994). The psychotropic 2C-B [2-(4-bromo-2,5-dimethoxyphenyl)-ethylamine, known on the street as "Nexus" or "Cyber"] behaves as a partial agonist toward both α_1-adrenergic and 5-$HT_{2A/2C}$ serotonergic receptors (apparent pD_2^S and pD_2^A, 7.54 and 5.24, respectively) (Lobos *et al.*, 1992), in agreement with its approximately 35 nM K_i in the nonselective displacement of both [^{3}H]-ketanserin (5-HT_{2A}) and [^{3}H]-mesulergine (5-HT_{2C}) (Glennon *et al.*, 1992). On the other hand, the much less potently psychoactive 2C-N [2-(2,5-dimethoxy-4-nitrophenyl)-ethylamine] does not elicit aortal contraction and antagonizes the responses to norepinephrine (NE, apparent pA_2=5.98) or serotonin (5-HT). A study of the behavior of several other phenylethylamines in the rat thoracic aorta preparation led to the suggestion that α_1-adrenergic activities, together with possibly weak or partial 5-HT_2 agonistic activity or even antagonism, might contribute to the effects reported for some of these substances in humans (Sáez *et al.*, 1994).

To determine whether β-alkoxylation of phenylethylamines mimics NE to any extent, we carried out a similar study on one β-ethoxy- and on a series of β-methoxyphenylethylamines (Torres *et al.*, 1995), bearing the same ring substituents as the previously studied analogues lacking the β-alkoxy group (Sáez *et al.*, 1994) and including those for which subjective reports are available from human volunteers (Lemaire *et al.*, 1985).

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TABLE 1. pD_2 values and relative maximal responses of variously substituted phenylethylamines

Substance	$pD_2 \pm SD$	Relative maximal response 5-HT	Relative maximal response NE	Substance	$pD_2 \pm SD$	Relative maximal response 5-HT	Relative maximal response NE
BOB-2	6.76 ± 0.11	0.73	0.55	2C-B[a]	4.55 ± 0.11	—	—
BOT-7	6.53 ± 0.05	0.67	0.52	—	—	—	—
BOI	6.52 ± 0.10	0.71	0.54	—	—	—	—
BOET	6.26 ± 0.11	0.47	0.36	—	—	—	—
BODMPEA	6.22 ± 0.03	1.03	0.79	2C-H[b]	6.74 ± 0.50	1.00	0.89
BOB	6.21 ± 0.06	0.75	0.56	2C-B[a]	4.55 ± 0.11	—	—
BOD	5.31 ± 0.06	0.47	0.36	2C-D[b]	5.06 ± 0.02	0.50	0.44
BOTMPEA	4.91 ± 0.11	0.84	0.64	TMPEA[b]	5.83 ± 0.18	0.42	0.36
BOM	4.73 ± 0.08	0.72	0.55	—	—	—	—
BOH	4.56 ± 0.12	0.85	0.65	MDPEA[b]	4.19 ± 0.12	0.49	0.42
—	—	—	—	DMPEA[b]	4.46 ± 0.21	0.39	0.33

Abbreviations: BOB-2, contains a β-ethoxy group; BOT-7, propylthio analogue; BOI, iodo analogue; BOET, contains an ethyl group; BODMPEA, a hydrogen atom replaces bromine; BOET, an ethyl group replaces bromine; BOB, contains a β-methoxy group.

[a] Lobos *et al.* (1992).

[b] Sáez *et al.* (1994).

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 300–350 g were killed by cervical contusion. The thoracic aorta was removed, freed from adjacent tissue and cut into 4–5 mm sections. Each piece of aorta was rubbed to remove the endothelium and then suspended horizontally between a pair of surgical stainless steel supports, one fixed to the bottom of the chamber and the other connected to the transducer, in a 25-ml tissue chamber containing modified Krebs-Henseleit solution with the following composition (mM): NaCl 112.0; KCl 4.7; $CaCl_2$ 2.0; $MgCl_2$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 15.0; glucose 11.5; and EDTA 0.026. The solution was kept at 37°C and oxygenated with a 95% O_2–5% CO_2 mixture, and the tissue sections were allowed to equilibrate for 2–3 hr under a resting tension of 3 g.

Cumulative dose–response curves were obtained by step-by-step increases in the concentration of NE, 5-HT or the test compound, adding 100-μl aliquots of appropriate solutions of these substances. More drug was added as soon as a steady response was obtained from the preceding dose, until no further contraction was observed. The results were reduced to Hill plots from which apparent pD_2 values were calculated. Analogous dose–response curves were obtained in the presence of either prazosin (10^{-8} M) or ketanserin (10^{-8} M), and the data were treated similarly to obtain apparent serotonergic (pD_2^S) and adrenergic (pD_2^A) affinities.

In regard to BON, which gave no contractile response, dose–response curves were obtained for 5-HT or NE in the presence of BON concentrations in the 10^{-7}–10^{-5} M concentration range, and the results were used to calculate the apparent serotonergic (pA_2^S) and adrenergic (pA_2^A) affinities.

The synthesis of the β-alkoxylated phenylethylamines used in this work has been described elsewhere (Torres *et al.*, 1995). NE [(−)arterenol bitartrate salt] and 5-HT creatinine sulfate were purchased from Sigma. Prazosin and ketanserin tartrate were kindly provided by Pfizer and by Janssen Pharmaceutica, respectively. All other chemicals were of analytical grade.

RESULTS

Table 1 summarizes the pD_2 values calculated for each compound, with the exception of BON, from the corresponding Hill plots (not shown), and the maximal responses relative to NE and 5-HT. For the sake of comparison, the published data for five structurally related phenylethylamine analogues with the same substitution patterns on the benzene ring are included. Table 2 repeats the pD_2 values for the β-alkoxylated phenylethylamine agonists, with their respective activity ranges, which indicate the lowest concentrations at which any contractile activity was observed and the concentrations at which the responses began to fall off, and gives the apparent serotonergic (pD_2^S) and adrenergic (pD_2^A) affinities calculated for each compound from experiments carried out in the presence of 10^{-8} M prazosin or ketanserin, respectively.

BOB-2, the only member of this set with a β-ethoxy group, is a 2C-B analogue and is the most potent compound in the series, showing an apparent pD_2 value slightly higher than that calculated for 5-HT, although the maximal response that it elicits is significantly lower than those produced by either NE or 5-HT tested under the same conditions. BOB, the β-methoxy analogue of BOB-2 and 2C-B, is about three times less potent, although exhibiting virtually identical maximal responses. The BOB congeners with a hydrogen atom (BODMPEA) or an ethyl group (BOET) replacing the bromine atom are virtually equipotent with BOB, and the iodo and the propylthio analogues (BOI and BOT-7) show similar activity at somewhat lower concentrations. Of all these compounds, only BODMPEA is able to elicit the same maximal response as that of 5-HT in aortal rings; therefore, all the other substances, with the exception of the antagonist BON, behave as partial agonists compared with 5-HT and NE (Tables 1 and 2). The remaining compounds show qualitatively similar behavior, but at concentrations one or two orders of magnitude higher.

BON did not show any vasoconstrictive activity in this preparation. After the tissue was preincubated with 10^{-7}–10^{-5} M solutions of this substance, the control NE curve was shifted to the right without any obvious change in its slope or maximal effect (Fig. 1). BON also shifts the serotonin control curve to the right in an apparently parallel fashion, although in this case the maximal effect seems to be somewhat reduced (Fig. 2). Comparison of the NE control curve and the graph obtained after incubation with BON (10^{-7}–10^{-5} M) leads to an apparent pA_2^A value of 7.45. If competitive antagonism at $5\text{-HT}_{2A/2C}$ receptors is assumed, an apparent $pA_2^S = 7.08$ may be calculated. Schild correlations (not shown) for increasing concentrations of

TABLE 2. Active concentration ranges, pD_2, pD_2^S and pD_2^A values of β-alkoxyphenylethylamines of the BOX family

Substance	Active range, M	$pD_2 \pm SD$	$pD_2^S \pm SD^a$	$pD_2^A \pm SD^b$	$pD_2^S - pD_2^A$
2C-B[c]	—	4.55 ± 0.11	7.54 ± 0.24	5.24 ± 0.24	2.30
BOB-2	3×10^{-9}–3×10^{-5}	6.76 ± 0.11	—	—	—
BOT-7	3×10^{-8}–3×10^{-4}	6.53 ± 0.05	5.71 ± 0.26	4.72 ± 0.02	0.99
BOI	3×10^{-8}–3×10^{-5}	6.52 ± 0.10	5.93 ± 0.13	5.11 ± 0.11	0.82
BOET	1×10^{-7}–3×10^{-5}	6.26 ± 0.11	5.30 ± 0.10	4.61 ± 0.05	0.71
BODMPEA	3×10^{-8}–1×10^{-4}	6.22 ± 0.03	5.34 ± 0.07	5.27 ± 0.11	0.07
BOB	3×10^{-8}–3×10^{-5}	6.21 ± 0.06	6.08 ± 0.08	4.43 ± 0.09	1.65
BOD	1×10^{-7}–3×10^{-5}	5.31 ± 0.06	4.66 ± 0.11	4.35 ± 0.01	0.31
BOTMPEA	3×10^{-8}–1×10^{-4}	4.91 ± 0.11	4.40 ± 0.06	3.87 ± 0.01	0.53
BOM	1×10^{-6}–3×10^{-4}	4.73 ± 0.08	4.54 ± 0.14	3.94 ± 0.06	0.60
BOH	3×10^{-7}–3×10^{-4}	4.56 ± 0.12	4.22 ± 0.14	4.04 ± 0.04	0.18

[a] Apparent affinities for serotonergic receptors.
[b] Apparent affinities for adrenergic receptors.
[c] Lobos *et al.* (1992).

NE or 5-HT were nonlinear, as expected for antagonism of a population of heterogeneous receptors with similar affinities for the drug.

DISCUSSION

All the β-alkoxyphenylethylamines tested by us—with the exception of BON, which, in the rat thoracic aorta, behaves as an antagonist to NE and 5-HT—elicited concentration-dependent contraction of aortal rings. This situation parallels the behavior of the β-unsubstituted phenylethylamines, of which only 2C-N (the β-demethoxy analogue of BON) antagonizes aortal contractions elicited by NE or 5-HT.

BOB-2 and BOB are β-alkoxy derivatives of the fairly well studied 2C-B ("Nexus," "Cyber") (Lobos *et al.*, 1992). The apparent affinity of BOB for aortal receptors is similar to those of the 4-ethyl derivative BOET and the 4-unsubstituted BODMPEA and is surpassed in the β-methoxy series only by the iodo analogue BOI and the propylthio derivative BOT-7. Nevertheless, when pD_2^S values are compared, BOI, BOB and BOT-7 appear to have practically identical affinities for $5\text{-HT}_{2A/2C}$ receptors, with BODMPEA and BOET lagging behind. Unexpectedly, BOD was found to be about an order of magnitude less active than either its higher (BOET) or its lower (BODMPEA) C-4 homologue in the isolated thoracic aorta. BOH and BOM are nearly two orders of magnitude less potent than BOB in the aortal preparation.

The qualitative responses of aortal rings to all the β-alkoxyphenylethylamines belonging to the BOX series were similar to those recorded for their β-unsubstituted analogues, as may be seen in Table 1. Most of these substances are partial 5-HT/NE agonists. BODMPEA was our only β-alkoxy compound to display complete agonistic behavior, just like the correspondingly ring-substituted and β-unsubstituted DMPEA (Sáez *et al.*, 1994). BON differed from all the other members of the set in behaving as a 5-HT/NE antagonist, the same as its β-unsubstituted analogue 2C-N (Sáez *et al.*, 1994).

Phenylethylamines with the 2,4,5-trisubstitution pattern on the ring, regardless of the presence or absence of a β-alkoxy substituent, generally seem to exhibit higher pD_2 values than do their analogues with the 3,4-disubstitution patterns (the BOH/MDPEA pair and DMPEA), although this is less clear when the serotonergic and adrenergic components are resolved. This trend is in agreement with what has long been known about the relative psychotropic potencies of such compounds in volunteers and points to a direct correspondence between the present *in vitro* results and the subjective re-

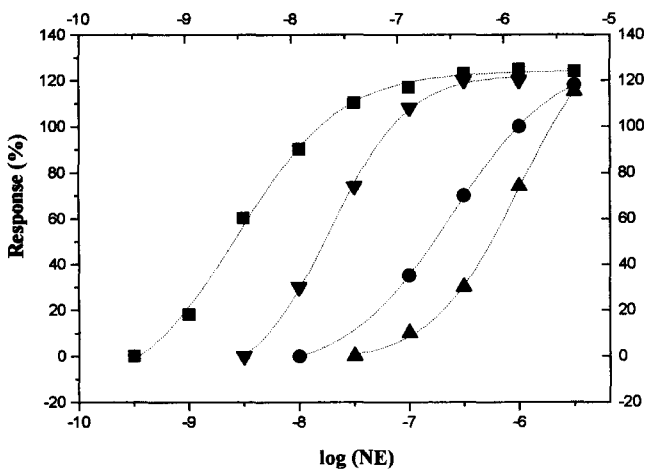

FIGURE 1. Response versus log dose curves for NE in the absence (■) and in the presence of BON [10^{-7} M (▼), 10^{-6} M (●), 10^{-5} M (▲)].

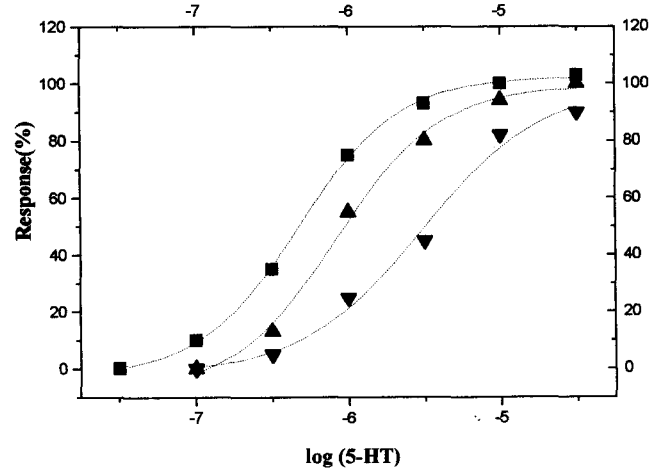

FIGURE 2. Response versus log dose curves for 5-HT in the absence (■) and in the presence of BON [10^{-6} M (▲) and 10^{-5} M (▼)].

ports on the oral activity of phenylethylamines and amphetamine analogues. A somewhat puzzling exception to this correspondence is the 2C-H/BODMPEA pair. Both compounds, with a 2,5-substitution pattern on the ring, show relatively high pD_2 values besides eliciting the greatest maximal response relative to NE and 5-HT.

The exceptional behavior of the 4-nitro compound BON, the only member of the BOX series to act as a serotonergic and adrenergic antagonist, finds its counterpart in its phenylethylamine analogue 2C-N. Apparent pA_2 values obtained from cumulative dose–response curves in the presence of 10^{-7}, 10^{-6} or 10^{-5} M concentrations of BON (Fig. 1) or 2C-N show that the β-methoxy compound ($pA_2^A = 7.45 \pm 0.18$) is a more effective antagonist to NE than the phenylethylamine derivative (5.98 ± 0.09) (Sáez *et al.*, 1994). It might be of interest to establish a direct comparison of the basic pharmacology of 2C-N with that of BON, because these compounds appear to be the sole examples so far of simple phenylethylamines that behave as α_1-adrenergic and 5-$HT_{2A/2C}$-serotonergic antagonists. Similar studies of the identically ring substituted, potent hallucinogen DON [(±)-1-(2,5-dimethoxy-4-nitrophenyl)-2-aminopropane] (Gómez-Jeria *et al.*, 1987) also are warranted.

Both series of phenylethylamines are nonselective, and their contractile activity indicates a mixture of serotonergic and adrenergic effects. In the β-alkoxy series, for which apparent pD_2^S and pD_2^A values were obtained, affinities are in general two- to eightfold greater for serotonergic than for adrenergic receptors, although the ring-disubstituted BODMPEA and BOH are virtually nonselective (Table 2). This poor selectivity contrasts with the purported strong preference of hallucinogenic phenylisopropylamines for 5-HT_2 receptors (Glennon *et al.*, 1994) and is reminiscent of the less-selective character of lysergic acid diethylamide and some of its congeners (Watts *et al.*, 1995). For the sake of comparison, the β-unsubstituted 2C-B has been reported to show a 200-fold preference for 5-$HT_{2A/2C}$-serotonergic over α_1-adrenergic receptors (Lobos *et al.*, 1992). We found a selectivity ratio of about 40 for BOB, suggesting that β-alkoxylation may actually enhance adrenergic relative to serotonergic agonist behavior, as apparently intended when the first members of the BOX series were synthesized (Lemaire *et al.*, 1985; Shulgin and Shulgin, 1991). In human volunteers, BOB appears to be approximately equipotent with 2C-B (Lemaire *et al.*, 1985; Shulgin and Shulgin, 1991) in spite of its considerably lower affinities for both 5-$HT_{2A/2C}$ and α_1 receptors. Although the relatively strong adrenergic activity of BOD and BOH may be a contributing factor to the psychotropic syndrome elicited by them, it also seems possible that the adrenergic component, when its intensity approaches that of the serotonergic component, is responsible for unleashing the peripheral effects suspected for these drugs (Shulgin and Shulgin, 1991).

The presence of a stereogenic center at the β position and the fact that all the compounds of the BOX series tested thus far are racemic raise the question of the presumably different activities of the enantiomers at both the α_1 and the 5-$HT_{2A/2C}$ receptors. At this point, the possibility that one stereoisomer or the other may show enhanced selectivity is at best speculative. Nevertheless, because several of these compounds show better than micromolar pD_2 values, it may be of interest to attempt their resolution and compare the individual isomers in the same preparation.

SUMMARY

The contractile activity of 11 β-alkoxyphenylethylamines was assayed in the isolated rat thoracic aorta. All but BON elicited concentration-dependent contraction of this preparation, BOB-2 being the most and BOH the least potent. BON behaved as a competitive antagonist to NE and as a possible noncompetitive antagonist to 5-HT receptors with similar pA_2 values at both receptor types. All the other members of this family behaved as partial serotonergic and adrenergic agonists, with the exception of BODMPEA, which acted as a 5-HT complete agonist. β-Alkoxyphenylethylamines with a 2,4,5-substitution pattern on the aromatic ring were in general found to be more potent in this preparation than 3,4-disubstituted analogues. Resolution of the serotoninergic and adrenergic components of the contractile activity of these compounds showed that most of them were virtually nonselective, with the exception of BOB, which displayed about 40-fold selectivity for 5-$HT_{2A/2C}$ over α_1 receptors.

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