

THE ACTION OF THE PSYCHOACTIVE DRUG 2C-B ON ISOLATED RAT THORACIC AORTA

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Abstract—1. 2C-B [2-(4-bromo-2,5-dimethoxyphenyl)ethylamine] elicits concentration-dependent contraction of the rat thoracic aorta (apparent $pD_2 = 4.55$). The maximal contraction (E_{max}) attained with 2C-B is less than that produced by either norepinephrine (NE) or serotonin (5-HT).

2. Pretreatment with either prazosin (5×10^{-9} – 10^{-8} M) or ketanserin (5×10^{-9} – 10^{-8} M) leads to decreased slopes and E_{max} in the 2C-B dose–response curves.

3. 2.82×10^{-5} M 2C-B potentiates the response to low concentrations of NE; 5×10^{-5} M 2C-B shows similar behaviour, but with reduced E_{max} . At 10^{-6} M 2C-B acts as a competitive 5-HT antagonist; at 2.8×10^{-5} M, however, it behaves like a non-competitive 5-HT antagonist.

4. Removal of the endothelial lining from the aortal rings only shifts the 2C-B dose–response curve to the left.

5. These results suggest that 2C-B behaves as a partial agonist toward both α_1 -adrenergic and 5-HT₂ serotonergic receptors. The endothelium only seems to act as a diffusional barrier to the drug.

INTRODUCTION

2C-B [2-(4-bromo-2,5-dimethoxyphenyl)ethylamine] is a rather potent psychoactive analogue of mescaline, effective orally in humans at doses about 30 times lower than its natural prototype (Shulgin and Carter, 1975) but with a qualitatively different subjective profile (Shulgin and Shulgin, 1991). The lack of information suggesting its pharmacologic similarity to any abused compounds, providing evidence of abuse potential, or indicating the existence of actual abuse (Cicero *et al.*, 1986) has led to its classification as an uncontrolled substance by the WHO (Woods, 1986). Perhaps due to this circumstance, as well as the fall out of scientific fashion of compounds which alter perception in normal subjects, the pharmacology of 2C-B is virtually unknown aside from the transient psychological changes it elicits.

Although present knowledge of the psychopharmacology of 2C-B does not allow it to be clearly classed as a stimulant, a hallucinogen or an entactogen (Nichols, 1986), related drugs are usually active at 5-HT₂ and α_1 -adrenergic receptors (Luscher and Vanhoutte, 1988). The rat thoracic aorta is an easily accessible peripheral model rich in both α_1 (Cohen *et al.*, 1986) and 5-HT₂ receptors (van Nueten *et al.*, 1984), and thus appears to be a potentially useful screen for psychoactive substances of this kind.

In this study we report the contractile effect of 2C-B on the rat thoracic aorta, its interactions with the selective α_1 -adrenergic antagonist prazosin and the selective 5-HT₂ selective antagonist ketanserin, and with norepinephrine (NE) and serotonin (5-HT), and the effect of removal of the endothelial lining from the aortal segments on their sensitivity to 2C-B.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 300–350 g were killed by cervical contusion. The thoracic aorta was removed, freed of adjacent tissue, and cut into 5 mm sections. Each piece of tissue was suspended horizontally in a 10 ml tissue chamber containing modified Krebs solution (Cohen *et al.*, 1986) between a pair of surgical stainless steel supports, one fixed to the bottom of the chamber and the other connected to the transducer. The solution was kept at 37°C and oxygenated with a 95% O₂–5% CO₂ mixture, and the tissue rings were allowed to equilibrate for 2 hr under a resting tension of 4 g. The endothelial lining was removed for some experiments by gently rubbing the lumen of the aortal segments with a thin wooden rod (Cocks and Angus, 1983).

Cumulative dose–response curves were obtained by step-wise increases in the concentration of NE, 5-HT, or 2C-B, adding 100 μ l 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. Dose–response curves to NE and 2C-B were also obtained after incubating the tissue for 30 min with prazosin (selective α_1 -adrenergic antagonist, 10^{-9} – 10^{-7} M). Additional curves were similarly obtained with NE, 5-HT and 2C-B after incubation with ketanserin (selective 5-HT₂ antagonist, 5×10^{-9} – 5×10^{-7} M). The results were reduced to Hill plots from which apparent pD_2 values were calculated.

Prazosin and ketanserin tartrate were kindly supplied by Pfizer and by Janssen Pharmaceutica, respectively. 2C-B hydrobromide was synthesized in our laboratory following the literature (Shulgin and Carter, 1975) (structure confirmed by ¹H NMR) and appeared pure on TLC. NE ((–)-arterenol bitartrate salt) and 5-HT creatinine sulfate were from Sigma.

RESULTS

Figure 1 shows a typical sigmoidal dose–response curve to 2C-B in aortal rings, in the 10^{-5} – 5×10^{-4} M

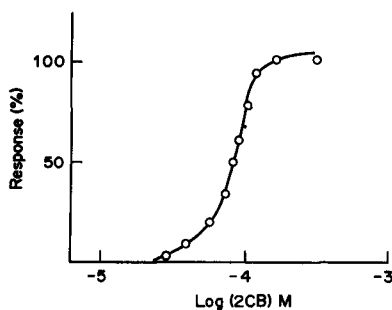


Fig. 1. Cumulative concentration-response curve to 2C-B in rat thoracic aortal rings. The response to each cumulative dose is expressed as percentage of the maximum contractile response achieved by the drug. Doses are expressed as the log of the final molar concentration in the bath. This curve represents a single case.

concentration range. The Hill plot of these data led to an estimate of $pD_2 = 4.55 \pm 0.11$ for 2C-B. Similar experiments with NE and 5-HT led to apparent pD_2 values of 7.54 ± 0.24 and 5.24 ± 0.24 , respectively. The maximal contractions (E_{max}) attained with 2C-B were consistently less than those observed with either neurotransmitter.

The effects of increasing concentrations of prazosin (10^{-9} – 10^{-8} M) on the dose-response curves to 2C-B are depicted in Fig. 2. 10^{-9} M prazosin had no significant effect, but higher concentrations led to reductions of E_{max} by about 40 and 70%, respectively. Figure 3 shows the effects of different concentrations of ketanserin (5×10^{-9} – 5×10^{-8} M) on the dose-response curves to 2C-B. In these experiments, E_{max} fell by about 30–60% as the antagonist concentration was increased, with clearly visible reduction of the slope.

When the aortal preparation was preincubated with 2.82×10^{-5} M 2C-B (corresponding to its apparent pD_2), the initial segment of the dose-response curve to NE appeared elevated with no appreciable change at higher NE concentrations, as can be seen in Fig. 4. Upon doubling the concentration of 2C-B, a synergistic effect was noticeable at low NE concentrations, but it was clearly smaller than at 2.82×10^{-5} M, and in the upper range the slope of the curve was reduced and E_{max} was depressed by about 25%. Similar experiments with 5-HT (Fig. 5) showed a non-competitive 5-HT antagonist be-

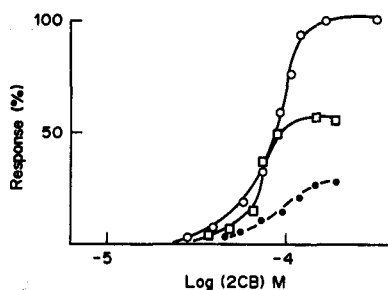


Fig. 2. Cumulative concentration-response curves to 2C-B in rat thoracic aortal rings obtained in the absence (○) and presence of prazosin (5×10^{-9} M, □; 10^{-8} M, ●). With 10^{-9} M prazosin the dose-response curve was indistinguishable from the control. Each curve represents a single case.

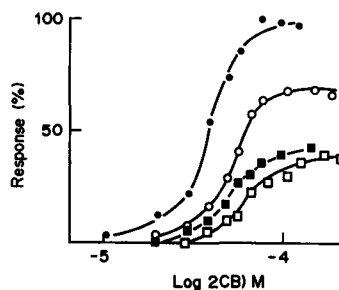


Fig. 3. Cumulative concentration-response curves to 2C-B in rat thoracic aortal rings obtained in the absence (●) and presence of ketanserin (5×10^{-9} M, ○; 10^{-8} M, ■; 5×10^{-8} M, □). Each curve represents a single case.

haviour of 2.82×10^{-5} M 2C-B. At 10^{-6} M, however, this drug seemed to act as a competitive antagonist, shifting the 5-HT dose-response curve to the right with no change in the E_{max} .

The influence of the endothelial lining on the sensitivity of aortal segments to 2C-B may be seen in Fig. 6. Rubbed rings produced a dose-response curve displaced to the left of the control curve, with no change in slope or E_{max} . Figure 7 shows the effect of endothelium removal on the activity of 2C-B in the presence of 5.0×10^{-8} M ketanserin. In this case, a similar pseudoparallel displacement of the dose-response curve can also be seen, with reduced slope and E_{max} .

DISCUSSION

The results obtained in the evaluation of the activity of 2C-B on rat thoracic aorta (Fig. 1) show that this molecule stimulates contraction of the vascular smooth muscle in the concentration range studied, although the E_{max} values obtained are always lower than with NE or 5-HT, which also elicit contraction in this preparation, indicating that 2C-B is only a partial agonist. At the same time, its apparent pD_2 of 4.55 shows that it is significantly less potent than either of these neurotransmitters.

The dose-response curve for 2C-B in the presence of a low concentration of the selective α_1 -adrenergic blocker prazosin (10^{-9} M) does not show any significant changes from the control. Nevertheless, when prazosin concentration is raised five- or ten-fold, the maximal response is depressed by about 40 and 70%,

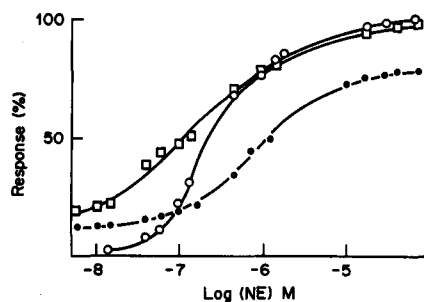


Fig. 4. Cumulative concentration-response curves to NE in rat thoracic aortal rings obtained in the absence (○) and presence of 2C-B (2.8×10^{-5} M, □; 5×10^{-5} M, ●). Each curve represents a single case.

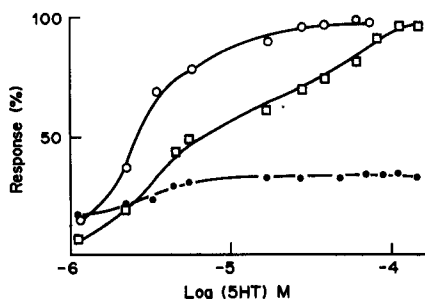


Fig. 5. Cumulative concentration-response curves to 5-HT in rat thoracic aortal rings obtained in the absence (○) and presence of 2C-B (10^{-6} M, □; 2.8×10^{-5} M, ●). Each curve represents a single case.

respectively, with a clear decrease of the slope at 10^{-8} M antagonist (Fig. 2). A similar analysis of the effects of the selective 5-HT₂ blocker ketanserin on 2C-B dose-response curves showed that this antagonist also reduces the maximal response to 2C-B and the slope of the curve in the 5×10^{-9} – 10^{-8} M range (Fig. 3). These results suggest that 2C-B, at concentrations approaching 10^{-4} M, exerts its agonistic actions at different sites from those occupied by α_1 -adrenergic and 5-HT₂ antagonists, although the response may be modulated by occupation of these receptors. As prazosin is more effective than ketanserin in reducing the maximal response to 2C-B, it seems reasonable to assume that the vascular activity of this drug is largely regulated by α_1 -adrenergic receptors.

At a concentration corresponding to its apparent pD_2 (2.8×10^{-5} M), 2C-B enhances the effect of NE on aortal rings, particularly at lower NE concentrations (Fig. 4). When the 2C-B concentration is raised to 5.0×10^{-5} M, however, a dualist behaviour emerges, with some synergy at $<10^{-7}$ M NE and clear non-competitive antagonism at higher NE concentrations. The interaction of 2.8×10^{-5} M 2C-B with 5-HT corresponds to a non-competitive antagonism, but with 10^{-6} M 2C-B this substance behaves more like a competitive antagonist (Fig. 5).

Removal of the endothelial lining of the aortal segments led to a pseudoparallel shift of the 2C-B dose-response curves, with no change in the maximal contraction (Fig. 6). This suggests that the site of

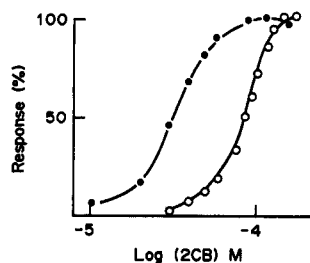


Fig. 6. Effect of endothelium removal on the 2C-B dose-response curve in rat aortal rings. The response to each cumulative dose is expressed as percentage of the maximum contractile response achieved with the drug. Doses are expressed as the log of the final molar concentration in the bath. This curve represents a single case. Rings with intact endothelium (○); without endothelium (●).

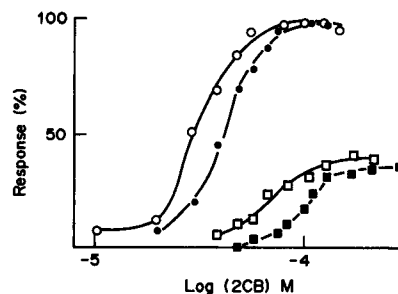


Fig. 7. Cumulative concentration-response curves to 2C-B in rat thoracic aortal rings in the absence and presence of ketanserin. The curves were obtained using intact and rubbed aortal segments. Control with intact endothelium (○); 5×10^{-8} M ketanserin and intact endothelium (□); control without endothelium (●); 5×10^{-8} M ketanserin without endothelium (■). Each curve represents a single case.

action of the drug is not in the endothelium, but that this tissue acts as a barrier to diffusion of 2C-B to receptors present in the smooth muscle. The dose-response curves for 5-HT in rubbed and un-rubbed aortal rings show similar behaviour. Experiments carried out with 5-HT in the presence of 2.5×10^{-8} M ketanserin indicate that in these cases removal of the endothelial lining increases sensitivity to the neurotransmitter, but still produces a pseudoparallel shift of the dose-response curve upon adding the antagonist (Fig. 8). When analogous experiments are carried out with 2C-B instead of 5-HT, the E_{max} and slope of the curve are affected similarly by ketanserin regardless of the presence or absence of endothelium (Fig. 7). In conclusion, this tissue does not seem to interfere with the interaction of ketanserin with the aortal 5-HT₂ receptors activated by both serotonin and 2C-B.

SUMMARY

2C-B causes concentration-dependent contraction of the rat thoracic aorta in the 10^{-5} – 5×10^{-4} M range, with $pD_2 = 4.55$, significantly lower than the pD_2 values determined for NE and serotonin 5-HT. The E_{max} attained with 2C-B is less than that

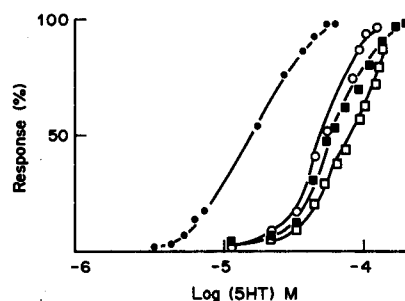


Fig. 8. Cumulative concentration-response curves to 5-HT in rat thoracic aortal rings in the absence and presence of ketanserin. The curves were obtained using intact and rubbed aortal segments. Control with intact endothelium (○); 2.5×10^{-8} M ketanserin and intact endothelium (□); control without endothelium (●); 2.5×10^{-8} M ketanserin without endothelium (■). Each curve represents a single case.

produced by either NE or 5-HT. Pretreatment with either prazosin (10^{-9} – 10^{-8} M) or ketanserin (5×10^{-9} – 5×10^{-8} M) gives rise to decreased slopes and E_{\max} in the 2C-B dose–response curves. 2.82×10^{-5} M 2C-B potentiates the response to low concentrations of NE, and does not modify E_{\max} . At 5.0×10^{-5} M, 2C-B shows similar synergistic behaviour but E_{\max} is reduced by 25%. 2.82×10^{-5} M 2C-B behaves as a non-competitive 5-HT antagonist, depressing E_{\max} by 70%. At 10^{-6} M, 2-CB acts like a competitive antagonist with regard to 5-HT. Removal of the endothelial lining from the aortal segments does not modify E_{\max} , but shifts the 2C-B dose–response curve to the left. These results suggest that 2C-B behaves as a partial agonist toward both α_1 -adrenergic and 5-HT₂ serotonergic receptors, although it may not be acting at the same sites as prazosin and ketanserin. The role of the endothelium in connection with the action of 2C-B appears to be merely as a barrier to diffusion of the drug to its receptors.

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