THE SITE AND THE MECHANISM OF PHENOXYBENZAMINE POTENTIATION OF THE PRESSOR RESPONSE TO OXYTOCIN AND VASOPRESSIN: IN VIVO AND ISOLATED AORTIC STRIPS STUDIES

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ABSTRACT


In intact rats, alpha adrenergic receptor blocking agents, phenoxybenzamine (PBZ) and phentolamine markedly potentiated the pressor response to oxytocin and vasopressin (VP). In the presence of PBZ or phentolamine blockade, the dose-response curves of oxytocin and VP were shifted to the left, resulting in an apparent doubling of the pressor potency of the neurohypophysial peptides. The beta adrenergic blocking agent, propranolol had no significant effect on the pressor response to oxytocin or VP. Combined PBZ and propranolol blockade did not alter the potentiating activity of PBZ. The PBZ-potentiating effect seems to be specific to the neurohypophysial peptides, since the pressor response to angiotensin was not potentiated by PBZ. The potentiating effect of PBZ on VP could be demonstrated in isolated rat aortic strips. This clearly indicates that the site of action of PBZ is directly on the vascular smooth muscle. However, in aortic strips, the effect of PBZ was seen only if norepinephrine (NE) was also added to the bathing medium. NE alone had no significant effect on the contractile response to VP. The requirement of both PBZ and NE for the potentiating effect suggests that an interaction between these two agents is involved in the PBZ potentiation of VP response. The possibility that NE and not PBZ is the direct agent causing the potentiation of VP response is discussed.

The neurohypophysial hormones, oxytocin (OXY) and vasopressin (VP), possess a marked vasopressor activity in rats (Van Dyke et al., 1955; Sawyer, 1961; Chan and du Vigneaud, 1962). The pressor response to these peptides is independent of the adrenergic system. Alpha adrenergic blocking agents of the haloalkylamine type do not antagonize the pressor responses to OXY and VP; on the contrary, these blocking agents have been found to enhance

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their responses (Dekanski, 1952; Supek et al., 1962). This potentiating effect of alpha adrenergic blocking agents on the pressor responses to VP was first effectively utilized by Dekanski (1952) to improve the sensitivity of the bioassay of VP in rats. The potentiating effect of phenoxybenzamine (PBZ) has since been incorporated into the official assay of VP activity by the U.S. Pharmacopeia. The site and mechanism of the potentiating action of PBZ have not been established, although there are reports which suggest a direct action on the vascular smooth muscle (Altura, 1967, 1970; Leenen and de Jong, 1969). We, therefore, sought to further ascertain the site and mechanism of this interaction between PBZ and OXY and VP. Portions of the preliminary work have been reported elsewhere (Erker and Chan, 1973, 1974).

Our investigations clearly establish the fact that the potentiating effect of PBZ on the pressor response to OXY and VP is a direct effect on the vascular smooth muscle with no significant cardiac involvement. The effect seems to be specific to neurohypophysial peptides. On the isolated rat aorta, the PBZ action requires the participation of the alpha adrenergic agonist norepinephrine (NE).

**Materials and Methods**

*In vivo experiments.* Male Sherman albino rats weighing between 220 and 370 g were used. The rats were anesthetized with urethane given in two doses, 1.0 mg/kg i.p. and 0.75 mg/kg s.c., and were maintained at approximately normal body temperature by an overhead lamp. Anesthesia was maintained by supplementary doses of urethane given s.c. when needed. Atropine, 1.0 mg/kg s.c., was also administered. Tracheotomy was performed. The right carotid artery was cannulated and the blood pressure measured with a Statham pressure transducer and monitored on a polygraph. The right jugular vein was cannulated for i.v. injections. All injections were made i.v. in 0.9% NaCl at 20-minute intervals or longer. The total volume injected never exceeded 0.25 ml.

*In vitro experiments—Aortic strip preparations.* The rat was stunned by a cranial blow and exsanguinated from the jugular veins. The abdomen was incised and the whole length of the abdominal aorta removed. The aorta was then dissected clean of connective tissues and cut into a spiral strip as described by Furchgott (1960). All strips were approximately 2 mm wide and 28 mm long. The aortic strips were set up for isotonic contractions recorded with the Harvard heart/smooth muscle isotonic transducer and transcribed on a polygraph. The base-line tension applied was 1.0 g. The bathing fluid used was the normal Krebs-Ringer bicarbonate solution aerated with 95% O₂-5% CO₂ and maintained at 37.5°C (composition in millimoles per liter: NaCl, 118; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; dextrose, 8.5). An equilibration period of 45 to 60 minutes was allowed before the experiment began.

Dose-response characteristics were studied by the cumulative dose-response curve technique as described by van Rossum (1953). All injections were made in 0.9% NaCl. The volume of injections was small so that when the maximal response was attained, the total volume injected would not increase the bath volume by more than 6%. At the ED₅₀ level the change in the bath volume was not considered significant and, therefore, correction was not made in calculating drug concentration in the bath.

In the NE pretreatment experiments the amine was added (10 ng/ml) after replacing the bathing medium with fresh Krebs-Ringer bicarbonate solutions. The NE was added four times at 12-minute intervals prior to determining the VP cumulative dose-response curve. The tissues contracted due to the NE and relaxed to base-line tension by the end of the 12-minute intervals. After the fourth and last addition of NE, the bathing medium was not changed. The cumulative dose-response curve to VP was determined after the fourth NE-induced contraction had subsided. When PBZ was introduced to the NE-treated tissue, it was always added only once immediately after the peak of the contraction induced by the third NE dose. The tissue was then washed. The fourth NE dose was given and the cumulative dose-response curve of VP studied as described above.

Statistical analysis. All responses were compared with the control responses. In *in vivo* experiments, either the animal served as its own control or a matched-pair control was used. In *in vitro* experiments, only matched-pair controls were used. All matched-pair control experiments were carried out simultaneously with the experimental counterparts. All data were analyzed statistically using the Student's *t* test for group comparisons (Snedecor, 1956). The occasional experiments which yielded data more than four standard deviations from the mean were rejected. Differences between means with a *P* value of .05 or less were considered significant.

**Drugs.** The peptides and drugs used were oxytocin (synthesized by du Vigneaud's Laboratory, Cornell University Ithaca, N.Y.), vasopressin (Pitressin, Parke, Davis and Company, Detroit, Mich.), angiotensin-II amide (Hypertensin, Ciba-Geigy, Corp., Summit, N.J.), phenoxybenzamine (Dibenzyline, Smith Kline and French Laboratories, Philadelphia, Pa.), phenylamine (Regitine, Ciba-Geigy, Corp.), propranolol (Inderal, Ayerst Laboratories, New York, N.Y.), norepinephrine (Levophed, Win-
throm Laboratories, Inc., New York, N.Y.), phenylephrine (Sigma Chemical Company, St. Louis, Mo.), isoproterenol (Isuprel, Winthrop Laboratories, Inc.), atropine sulfate and urethane. The above materials were either purchased or received as gifts.

Results

Effect of alpha adrenergic blocking agents on the pressor response to oxytocin and vasopressin. The effects of PBZ and phentolamine (PNT) on the pressor responses to OXY and VP were studied in in vivo anesthetized rats. Effective alpha adrenergic blockade was established with either PBZ (0.1-0.25 mg/kg i.v.) or PNT (10 mg/kg i.v., plus 4 mg/kg and 2.5 mg/kg at 80 and 160 minutes). These dose regimens of blocking agents produced and maintained a 60 to 90% inhibition of response to 0.1 mg/kg of phenylephrine over a 4-hour period, the duration of the experiment. These alpha adrenergic blocking dose regimens produced an average 26 mm Hg decrease in blood pressure.

Dose-response curves of OXY (at 3, 6, 12, and 24 mU/kg pressor units) and VP (at 12, 24, and 48 mU/kg) were determined in two groups of rats; each consisted of at least eight rats. The OXY group was treated with PBZ whereas the VP group was treated with PNT. Dose-response curves of OXY and VP were also simultaneously determined in matched-pair control animals, treated with only the saline vehicle.

Both PBZ and PNT potentiated the pressor responses to OXY and VP. In the presence of the alpha adrenergic blocking agent, the dose-response curves of OXY and VP were shifted to the left of the corresponding dose-response curves obtained from the matched-pair control animals. The shift of the curve resulted in an apparent doubling of the pressor potency of OXY or VP. Figure 1 shows the typical potentiating effect of the alpha blocking agent on the pressor response to the neurohypophysial peptide. In a separate series of experiments, where the animal served as its own control, the effects of PBZ and PNT were found to be interchangeable.

Effects of beta adrenergic blockade and combined alpha, beta adrenergic blockade on the pressor responses to oxytocin. The effect of the beta adrenergic blocking agent, propranolol (PRO) on the pressor responses to OXY was studied in a group of eight rats. Effective beta blockade was induced and maintained by PRO...
(2.5 mg/kg i.v. initially, at 90 and 180 minutes). This dose regimen of PRO caused only a slight decrease in the blood pressure of the rat, but produced an average 60% inhibition of the depressor response to isoproterenol (3.3 μg/kg i.v.) during the experimental period.

The dose-response curve of OXY (at 3, 6, 12 and 24 mU/kg) in rats treated with PRO was not statistically different from those of the matched-pair control animals treated only with the saline vehicle.

The effect of combined alpha, beta adrenergic blockade on the pressor response to oxytocin was studied in seven rats. As shown in figure 2, the simultaneous presence of PRO had no effect on the potentiating effect of PBZ on the pressor response to OXY.

Effect of PBZ on the pressor responses to angiotensin. The effect of PBZ on the pressor responses to angiotensin was studied in seven matched-pairs of rats. PBZ did not alter the pressor dose-response curve to angiotensin (0.1, 0.3 and 1.0 μg/kg).

Effects of PBZ on the contractile response of aortic strips to VP. Isolated rat aortic strips contract in response to VP in a dose-dependent manner. However, unlike the in vivo pressor responses, the contractile responses to VP were not affected by PBZ. In 10 aortic strips, exposure for 10 minutes to PBZ at 1.5 or 3.5 ng/ml markedly reduced the contractile response to 0.3 μg/ml of phenylephrine but the contractile responses to VP were not significantly altered from those of the control matched-pair tissues.

When the aortic strips were pretreated with NE (10 ng/ml) and then PBZ (1.5 ng/ml) as described under “Materials and Methods,” the VP dose-response curves of the PBZ-treated strips were shifted to the left of those of the matched-pair control tissues. The shift of the VP curve resulted in an apparent doubling of the potency of VP, an effect nearly identical with that observed in the in vivo experiments. Figure 3 shows results from seven aortic strips and their matched-pair control tissues.

Pretreatment with the NE alone (10 ng/ml) did not significantly affect the contractile response of the aortic strips to VP. The potentiating effect of VP was observed only in NE- and PBZ-treated tissues.

Pretreatment of aortic strips with isoproterenol neither caused significant relaxation of the
Effect of PBZ and OXY on the contractile response of aortic strips to vasopressin. Each curve represents the average values from seven aortic strips. The bars indicate the S.E.M. The treated strips were exposed to NE, 10 ng/ml, and PBZ, 1.5 ng/ml, as described under "Materials and Methods." The seven matched-pair control tissues were exposed only to NE. The dose-response curve of the NE-control tissues was not significantly different from that of the normal control tissues (not shown in the figure).

Effect of PBZ on the contractile responses of aortic strips to angiotensin. As was observed in the pressor response, NE and PBZ pretreatment did not affect the contractile response of aortic strips to angiotensin at doses of 0.1 to 100 ng/ml.

Discussion

Although it has been known for some time that haloalkylamine \textit{alpha} adrenergic blocking agents, such as PBZ, have a potentiating effect on the pressor response to OXY and VP, the site and the mechanism of this potentiating action have not been established. Our investigations show that the potentiating action of PBZ is not specifically related to its chemical structure, but most probably is related to its \textit{alpha} adrenergic blocking activity. This relationship is suggested by our findings that PNT, a substituted imidazoline possessing \textit{alpha} adrenergic blocking activity, also potentiated the pressor responses to VP (fig. 1). The doses of \textit{alpha} adrenergic blocking agents used in these experiments reduced the pressor responses to phenylephrine (100 μg/kg) to 10 to 40% of the control level. In the presence of this dose level of PBZ or PNT, the dose-response curves of OXY and VP were shifted to the left, resulting in an apparent doubling of the pressor potency of the peptides (figs. 1 and 2).

That PRO, in effective \textit{beta} adrenergic blocking doses, had little or no effect on either OXY-induced pressor responses or the potentiating effect of PBZ on the same (fig. 2) indicates a lack of \textit{beta} receptor involvement in the potentiation phenomenon. Leenen and de Jong (1969) had also found that PRO had no significant effect on the pressor response to a vasopressin analog, octapressin, in rats.

\textit{Alpha} adrenergic receptor blockade produced a decrease in blood pressure which was probably due to relaxation of the vascular smooth muscle cells. Since it has been shown that, within limits, the pressor response to vasoconstrictor agents frequently bears an inverse relationship to the initial blood pressure (Wilder, 1962), the enhanced pressor responses to OXY and VP in \textit{alpha} adrenergic blocked rats might simply be the result of a more relaxed vascular bed. However, our experiments with angiotensin indicate that PBZ potentiation of the pressor response to OXY and VP cannot be satisfactorily attributed to a more relaxed vascular bed alone because the pressor responses to angi-
neurohypophysial peptides.

Our demonstration that the potentiating effect of PBZ on VP can also be observed in isolated aortic strips provides additional evidence that relaxation of the vascular smooth muscle cells, consequent to α-adrenergic blockade, does not play a major role in producing the enhanced response to OXY and VP. In the isolated aortic strip preparation, the experimental tissue and its matched-pair control were always set and maintained at a constant basal tension of 1.0 g. Similar conclusion was drawn by Altura (1967, 1970) who studied the effects of various vasoactive substances on the microvascular system by direct microscopic observation and by Leenen and de Jong (1969) who studied VP pressor responses in pithed rats.

The demonstration of the potentiating effect of PBZ in isolated aortic strips clearly shows that the site of this PBZ action is directly on the vascular smooth muscle (fig. 3). Cardiac and vascular compensatory reflex involvements are not significant. This result is in accord with earlier work by Leenen and de Jong (1969) and Altura (1970). Thus, we conclude that the potentiating effect of PBZ on the pressor or the vascular smooth muscle contractile activity of OXY and VP represents a specific interaction at some cellular level between PBZ and the neurohypophysial peptides.

It may be argued that the pressor action of VP is primarily exerted on the small resistant vessels (Altura, 1967) and that the aorta, being relatively noncontractile, is atypical and may not be a suitable model. We believe that the difference, if it exists, between the responses of the great vessel and the small resistant vessels to VP is quantitative rather than qualitative. This belief is supported by our observations that the effects of PBZ and PRO on the contractile responses of the aortic strip to VP and angiotensin were practically parallel to those on the pressor responses observed in the intact animals.

It is important to note that PBZ potentiated the contractile response of the rat aortic strip to VP only when NE was also added to the bathing medium. The concentration of NE added to the bath was a contractile dose (10 ng/ml). This dose was intended to “restore” the sympathetic tone that is present in the intact animal when we found that PBZ alone had no effect on the contractile response of the aortic strip to VP. It is also important to point out that NE alone at this concentration had no significant effect on the responses of the aortic strips to VP. Potentiation of the responses of VP was observed only in tissues which were pretreated both with the NE and PBZ, suggesting that an interaction between these two agents is involved in the PBZ potentiation.

PBZ has been shown to alter the distribution and metabolism of NE through several actions which theoretically may have had a role in producing the potentiation phenomenon. PBZ has been shown to inhibit both the neuronal (Theonen et al., 1964) and extraneuronal uptake (Iversen, 1967) of NE as well as the access of NE to extraneuronal metabolizing enzymes (Langer, 1970). In addition, PBZ may increase the release of NE from sympathetic nerve terminals (Langer, 1970; Enero et al., 1972). The latter PBZ effect is probably irrelevant to our experiments on isolated aortic strips. By inhibiting NE uptake and metabolism, PBZ may thus increase the threshold NE response which is added to the VP response in the form of potentiation. The PBZ doses required to block neuronal and extraneuronal NE uptake and metabolism in in vitro preparations, including rat, have been in the range of 10 to 100 μg/ml (Avakian and Gillespie, 1968; Iversen and Langer, 1969; Langer, 1970; Enero et al., 1972), far in excess of the PBZ dose of 1.5 ng/ml which was used in the present experiments. However, this possible mechanism of PBZ potentiation cannot be excluded on this basis. The doses of PBZ used in our experiments were effective α-blocking doses and might have altered NE distribution and metabolism; although below chemically detectable level, it was biologically consequential.

Another possible mechanism may involve a common pathway in vascular smooth muscle contractions activated by both NE and VP. Bar telstone and Nasmyth (1965) had shown that nonpressor doses of VP potentiated the contractile response to NE in rat aortic strips. It is conceivable that in the presence of α-adrenergic receptor blockade, NE is freed from the adrenergic receptor sites to activate this pathway common to NE and VP. Thus, in intact
rats, PBZ blocks the pressor response to NE but potentiatates the response to VP. In isolated rat aortic strips, PBZ potentiation of the contractile response to VP can be demonstrated only when NE is also added to the bath.

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