VASCULAR DESENSITISATION - POSSIBLE ROLE OF PROSTAGLANDINS

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Summary : Prolonged exposure to noradrenaline (NA) brings about an increase in the release of prostaglandin (PG)-like material from rat aortic strip. The release is greater with oxymetazoline while methoxamine decreases it. These effects are blocked by yohimbine and prazosin respectively. Pretreatment with 6-OHDA or reserpine diminishes the release of PG-like material. Barium chloride, a non-specific spasmogen, does not affect the release significantly. It appears therefore that the source of PG-like material is presynaptic and that its release mechanism is linked to an alpha$_2$ (α_2) adrenoceptor. It is proposed that this release of PG-like material contributes to the development of desensitisation in vascular tissue.

Key words : adrenergic agonists prostaglandins desensitisation

INTRODUCTION

Prolonged exposure of a tissue to high concentration of an agonist, in course of time, renders that tissue less responsive to that corresponding agonist, This phenomenon of desensitisation has been documented in case of opiates (27, 12. 28, 21), cholinergic agonists and antagonists (29, 38), catecholamines and prostaglandins (32, 22, 11).

Desensitisation to catecholamines has been studied more extensively in respect of beta adrenoceptors (9, 14, 23, 4, 1). Recently, Omini *et al.* (26) have shown that in rat lung PG synthesis inhibition prevents the development of beta-adrenoceptor desensitisation. Similar results were obtained by Brink (8) in guinea-pigs. However, desensitisation has received much less attention with respect to alpha adrenoceptors (16, 10, 5). The present work deals wtih the desensitisation occuring in the alpha adrenoceptors of the vascular smooth muscle.

Abraham and Nasmyth (2) while working with interactions between infusions of NA and injections of vasopressin on rat aortic strip, observed that when phentolamine was infused along with NA, the development of desensitisation was blocked indicating that availability of alpha adrenoceptors was essential for the development of desensitisation. They further showed that desensitisation was more prominent when NA was replaced by a preferentially $alpha_2$ adrenoceptor agonist i.e. clonidine, than when NA was replaced by a preferentially $alpha_1$ adrenoceptor agonist like phenylephrine. This suggests that the alpha adrenoceptor involved in the process of desensitisation has the characteristics of $alpha_2$ adrenoceptor.

It has been reported that prostaglandin synthesis/release is increased under the influence of adrenergic agonists (24,40,30,17). Further, this is prevented by yohimbine but not prazosin (17).

Holmes *et al.* (19) have shown that PGs of E series antagonise the effect of vasoconstrictor substances like NA, vasopressin and angiotensin. There is also evidence to show that PGs are present and released from vascular wall and act as local modulators of vascular responses to various vasoactive substances (34). PGI_2 is known to inhibit the contractile responses of vascular tissue to exogenous NA as well as electrical stimulation (18). Indomethacin enhances the contractile responses to NA in animals (3,41) and human vascular tissue (7,18).

Putting the above facts together, there is every likelihood of PGs being released from the vascular wall as a result of continuous stimulation by adrenergic agonists, which in turn may reduce the responsiveness of blood vessel. To test this hypothesis we have carried out experiments to investigate :

1. Whether there is significant increase in the release of PGs from isolated rat aortic strip on continuous exposure to adrenergic agonists;
2. If yes, what is the source of these PGs and the mechanism of their release;
3. Whether the increase if any, is due to the mechanical response of effector cell i.e. contraction of aortic strip;
4. And lastly, whether the inhibition of PG synthesis influences the development of desensitisation in rat aortic strip.

MATERIAL AND METHODS

Animals : Albino rats of either sex, obtained from Haffkine Institute, Bombay and weighing 175 *g* - 250 *g* were used in this study. They were starved for 24 hr prior to the experiment. They were divided into 13 groups (Table I).

Bioassay method : The release of PG-like material by rat aortic strip was studied by a modified method of Rioux *et al.* (33). The animals were killed by a blow on the head and rapidly exsanguinated by cutting the carotid arteries. The aortic strip was prepared according to Furchgott and Bhadrakom (15). Each strip was stretched with a 1 *g* load suspended in 10 *ml* organ bath containing aerated tyrode solution at 37°C and incubated for a period of 90 min. Before each incubation the strips were washed 4 to 6 times over a 2 min period. At the end of incubation, the amount of PG-like material into the medium was estimated in terms of PGE_2 activity on rat fundus strips (37). The assay organs were equilibrated for 1 hr in a 10 *ml* organ bath filled with aerated tyrode solution at 37°C to which atropine, cyproheptadine, phentolamine, propranolol (all at a final concentration of 1×10^{-7} *g*) and indomethacin (1×10^{-6} *g*) were added to increase the specificity and sensitivity of the assay for PGs.

Drugs : The following drugs were used either alone or in combination to observe their effects on the release of PG-like material : NA 500 *ng/ml*, barium chloride 10 *μg/ml*, oxymetazoline 50 *ng/ml*, methoxamine 10 *μg/ml*, 6-OHDA 25 *μg/ml*, yohimbine 10 *ng/ml* and prazosin 20 *μg/ml*. Reserpine 5 *mg/kg* was administered i.p. 18 hr prior to the experiment.

In the case of first five, the aorta was perfused with the drug dissolved in tyrode solution for a period of 30 min, washed for 10 min in plain tyrode and later incubated for 90 min. Yohimbine and prazosin when used alone were added to the incubation medium. When a combination of yohimbine and oxymetazoline or prazosin and methoxamine was used, the aorta was kept in contact with the antagonist for 10 min and then perfused with the combination for 30 min.

Effect of PG synthesis inhibition on desensitisation : Experiments were also performed to observe the effect of PG-synthesis inhibition on the development of desensitisation. Responses of helical rat aortic strips were obtained to bolus doses of NA (300,600 and 900 *ng/ml*) prior to and after perfusion of rat aortic strip for 30 min with NA (500 *ng/ml*) in control (n=6), and animals treated with indomethacin 5 *mg/kg*, i.p. 24 hr prior (n=6). Contractions were recorded by using a force displacement transducer on a four channel polygraph (Encardiorite, India).

Statistical analysis : The results were analysed by Student's t-test.

RESULTS

PG release : Table I shows the PG-like material released in the incubation medium from control and treated aortic strips.

TABLE I : Effect of drugs and chemicals on the release of prostaglandin-like material from rat aortic strip.

S. No.	*Perfusion conditions*	*PG-like materialin ng/mg (mean ± S.E.)*	*P value*
1.	Control (6)	3.70±0.267	
2.	Noradrenaline (6)	6.20±0.378	<0.001*
3.	Barium Chloride (6)	3.49±0.550	N.S.
4.	Oxymetazoline (6)	7.94±0.364	<0,001*
5.	Yohimbine (4)	1.97±0.284	<0.01*
6.	Yohimbine + Oxymetazoline (6)	1.80±0.0284	<0.001**
7.	Methoxamine (6)	1.84±0.088	<0 001*
8.	Prazosin (4)	6.14±0.218	<0.001*
9.	Prazosin + Mathoxamine (4)	3.67±0.210	<0.001***
10.	6-OHDA (6)	0.20±0.044	<0.001*
11.	6-OHDA + Oxymetazoline (4)	1.29±0.145	<0.001**
12.	Reserpine (4)	1.40±0.025	<0.001*
13.	Reserpine + Oxymetazoline (6)	2.30±0.293	<0.001**

Figures within parenthesis indicate number of experiments.
* as compared to control.
** as compared to oxymetazoline group.
*** as compared to methoxamine group.
N.S. = not significant.

It can be seen that NA and oxymetazoline increased the release of PG-like material significantly whereas barium chloride did not affect it to a significant extent. Yohimbine, methoxamine, 6-OHDA and reserpine diminished the release significantly. The effect of oxymetazoline is not seen in the presence of yohimbine or 6-OHDA and in reserpinised tissue, while that of methoxamine is blocked by prazosin.

Effect of PG synthesis inhibition on desensitisation : The responses of rat aortic strip to bolus doses of 300, 600 and 900 *ng/ml* of NA were reduced significantly by 78,60 and 36% respectively after perfusion with NA in control animals while they were not significantly affected in those pretreated with indomethacin.

DISCUSSION

The phenomenon of desensitisation and the intricacies of its mechanism have attracted the attention of several groups of workers over a number of years. Among the many explanations put forward to explain the phenomenon of desensitisation, is the liberation of vasodilator substances like histamine (16). To this can be added yet another class of substances, the PGs, which are widely distributed, are present and released locally in the vascular tissue and are known to modulate the release of NA from the nerve endings as well as responses of vascular tissue. Their evanescent action, potency, local synthesis and metabolism in the tissues are additional desirable attributes for such a role. Staszewska-Barczak and Vane (37) have provided evidence to show that release of PGs from vascular wall is responsible for local modulation of actions of various vasoactive substances. Blumberg *et al.* (6) have shown that synthesis of a PGE_2 like material might be responsible for desensitisation observed in vascular tissues in response to vasoactive peptides like angiotensin I, angiotensin II and bradykinin. It is also known that PGs obtund the vasoconstrictor properties of NA and vasopressin (19). The results of our experiments reveal a significant increase in the release of PG-like activity from the aortic strip on prolonged exposure to NA and justifies a conclusion that release of PG-like material can be one important factor contributing to development of desensitisation in the vascular smooth muscle.

It can be seen from the results (Table I) that prolonged exposure to NA brings about an increase in the release of PG-like material from rat aortic strip. This is in agreement with the findings of Wennmalm & Brundin (40) in isolated rabbit heart and Pipili & Poyser (31) in rabbit mesenteric arterial bed.

When oxymetazoline, a preferentially presynaptic (α_2) adrenoceptor agonist, is used in lieu of NA, the release of PG-like material is substantially increased. This is in tune with the findings of Olsen (24) and Griffiths and Moore (17), wherein clonidine, a preferentially presynaptic (α_2) adrenoceptor agonist is shown to increase PG release in various tissues. Yohimbine, a specific blocker of presynaptic (α_2) adrenoceptors, by itself diminished the basal release of PG-like material and in addition significantly blocked the

release of oxymetazolin induced PG-like material. Griffiths and Moore (17) have also observed that clonidine induced stimulation of PG synthesis in rat vas deferens is blocked by yohimbine. From this experimental evidence it appears that the adrenergic agonist-induced release or PG-like material from the rat aortic strip is operated by a receptor which has properties similar to presynaptic (α_2) adrenoceptor.

When NA was replaced by methoxamine, a preferentially post-synaptic (α_1) adrenoceptor agonist, there was a significant decrease in the release of PG-like material. Wennmalm and Brundin (40) observed a similar decrease in PG release from isolated rabbit heart when methoxamine was infused. This suggests that the postsynaptic (α_1) adrenoceptor is inhibitory to the release of PGs. Further, in tune with the above argument, prazosin, a preferentially postsynaptic (α_1) adrenoceptor antagonist increased the outflow of PG-like material. This corroborates the finding of Pipili (30) that the stimulation induced PG release from perfused rabbit mesenteric arteries is increased in the presence of prazosin. Used with methoxamine, prazosin blocked the effect of methoxamine as expected.

Studies of Abraham and Nasmyth (2) with the interactions of NA and vasopressin on the rat aortic strip indicate that an alpha adrenoceptor is linked with the development of desensitisation. They further demonstrated that the adrenoceptor involved has characteristics of a presynaptic (α_2) adrenoceptor. Considering our results with PG release, together with the above observations of Abraham and Nasmyth (2), it is reasonable to postulate that the NA induced release of PG-like material from rat aortic strip is also linked with an alpha adrenoceptor and further, may be responsible for the development of desensitisation to NA.

The mechanism of the release of PGs by adrenergic agonists is incompletely known. Wennmalm and Brundin (40) have argued that the receptor controlling PGE release is different from the conventional alpha and beta adrenoceptors responsible for electromechanical and metabolic responses. They have labelled this receptor as "PG-adrenoceptor" which constitutes a single functional unit. It has been suggested by Omini *et al.* (25) and Griffiths and Moore (17) that activation of presynaptic (α_2) adrenoceptor brings about an increase in PG synthesis possibly through activation of phospholipase-A_2 and release of arachidonic acid from membrane phospholipids. Wennmalm and Brundin (40) and Pipili (30) maintain that the postsynaptic (α_1) adrenoceptor is inhibitory to the release of PGs though the exact mechanism of this inhibition is yet to be elucidated.

To rule out the possibility that the release of PGs is not specific to adrenergic agonists and is merely an expression of the physical process of contraction of the aortic strip, we did experiments employing barium chloride, a non-specific spasmogen that brings

about smooth muscle contraction. Barium chloride did not increase the release of PG-like material. Pipili and Poyser (31) using potassium chloride also observed that the PG release remained unchanged after the administration of this non-specific vasoconstrictor.

Regarding the origin of PGs released in autonomically innervated tissues, there is a plethora of evidence both in favour of effector cell i.e. postsynaptic site and nerve endings i e. presynaptic site. In order to locate the site from which this PG-like material is released, we conducted experiments with 6-OHDA, a drug known to destroy the adrenergic nerve terminals. Oxymetazoline failed to increase of PG-like material in 6-OHDA pretreated rat aortic strips. This observation is further corroborated by the findings in reserpinised rats, where once again oxymetazoline failed to enhance the basal release of PG-like material. These two observations indicate that the source of PG-like material is presynaptic.

Our experiments performed to observe the development of desensitisation to NA in rat aortic strips show that PG synthesis inhibition significantly obtunds the development of desensitisation.

In conclusion, the results of our experiments show that the release PG-like material from rat aortic strip is a specific process linked to presynaptic (α_2) adrenoceptor and that the source of this PG-like material is presynaptic. It is proposed that this release of PG-like material probably contributes to the development of desensitisation in vascular tissue. This hypothesis is supported by the recent findings of Omini *et al.* (26) that arachidonic acid metabolites have a role to play in β-receptor desensitisation.

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