VASOMOTOR ACTIONS AND SMOOTH MUSCLE RELAXANT PROPERTIES OF SOME INTERMEDIATES OF CHLORAMPHENICOL

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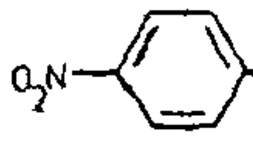
ABSTRACT

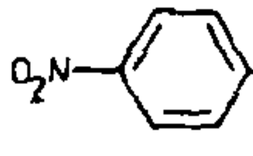
ω-Amino-*p*-nitroacetophenone hydrochloride (II), ω-acetamido-*p*-nitrophenone (III) and ω-acetamido hydroxymethyl-*p*-nitrophenone (IV) are intermediates formed in the synthesis of chloramphenicol from *p*-nitroacetophenone. The effects of these compounds on the blood pressure of anaesthetised cats and on the smooth muscles of different species of animals were studied to find structure-function relationship, if any, of chloramphenicol and its intermediates. When injected intravenously the compounds II and III produced a depression in blood pressure followed by an over-shooting rise. Using different blocking agents, it was postulated that the vasodepression was a direct effect of the compounds on vascular smooth muscle. The secondary vasopressor effect of the compounds was a sympathomimetic effect. The actions were similar to those observed with chloramphenicol and its hydrolytic product 2-amino-1-*p*-nitrophenyl propane-1,3-diol. The compound IV, however, had no cardio-vascular effect. All the compounds, however, inhibited smooth muscles of various species of animals.

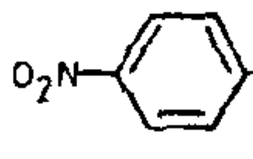
INTRODUCTION

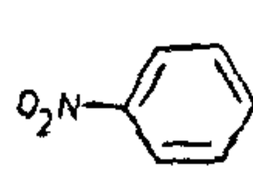
CHLORAMPHENICOL inhibited smooth muscles of laboratory animals (guinea pig ileum, guinea pig trachea, rabbit ileum, rabbit jejunum and rabbit aortic strip) decreasing both the height and frequency of spontaneous contractions. Chloramphenicol-induced relaxation was not mediated through adrenergic, cholinergic or histaminergic mechanisms and appeared to act directly on the muscle[1]. Chloramphenicol after hydrolysis is converted into 2-amino-1-*p*-nitrophenyl propane-1,3-diol. This compound also inhibited smooth muscles like chloramphenicol[2]. After intravenous administration, both chloramphenicol and the hydrolytic product produced vasodepression followed by an over-shooting rise of blood pressure[2]. Hypertension was a sympathomimetic effect[3].

The most efficient of the several routes devised for the synthesis of chloramphenicol commenced with *p*-nitroacetophenone[4]. The different compounds formed in the process are:

(I)  — (p — NITROACETOPHENONE)

(II)  — (ω — AMINO — p — NITROACETOPHENONE HYDROCHLORIDE)

(III)  — (ACETAMIDO FORM)

(IV)  — (HYDROXYMETHYL PRODUCT)

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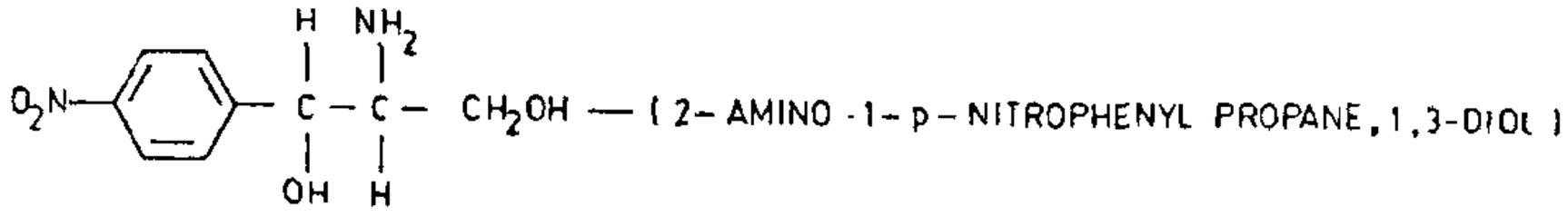
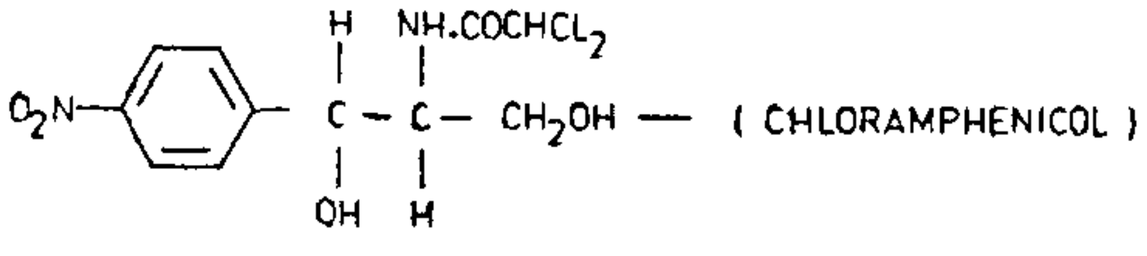
(V) 

(2– AMINO -1– p – NITROPHENYL PROPANE , 1 , 3-DIOL)

(VI) 

(CHLORAMPHENICOL)

It was our interest to study the effects of compounds II, III and IV on the blood pressure of anaesthetized cats and on different smooth muscles of animals in order to find structure-activity relationship, if any, of chloramphenicol and its intermediates.

Materials and Methods

Isolated smooth muscle experiments were undertaken with the guinea pig ileum[5], guinea pig tracheal chain[1], rabbit leium[1] and rabbit jejunum[3] preparations which were set up according to the standard procedures. The experimental designs with these smooth muscles had been the same as described earlier[1].

Adult cats of either sex, 2·5 to 4 kg, were anaesthetized with phenobarbital sodium, 150 mg/kg intramusculary. Standard pharmacological methods[5] were used for the cat spinal preparation and blood pressure recordings.

The compounds II, III and IV, dissolved in isotonic sodium chloride solution, were added to the organ bath fluid in smooth muscle experiments and injected through femoral vein in cat blood pressure experiments as described earlier[2].

Results

Smooth Muscle Effects of Compounds II, III and IV

Smooth muscle response with the above compounds were observed in guinea pig ileum, guinea pig trachea, rabbit ileum and rabbit jejunum preparations. For guinea pig ileum experiments (3 experiments for each compound) the agonists used were acetylcholine and histamine. Significant alteration in the height of the contraction of guinea pig ileum against both the agonists became evident only after a relatively high concentration of the compounds (80 μg/ml bath fluid). Incremental increases in concentrations of compounds (80-640 μg/ml bath fluid) produced progressive reductions in the height of contraction by the guinea pig ileum (Figs. 1 and 2).

In rabbit ileum experiments (3 experiments for each compound) the minimum concentration of the compounds required to inhibit the frequency and amplitude of spontaneously occurring rhythmic pendular contraction was 0·25 mg/ml of bath fluid.

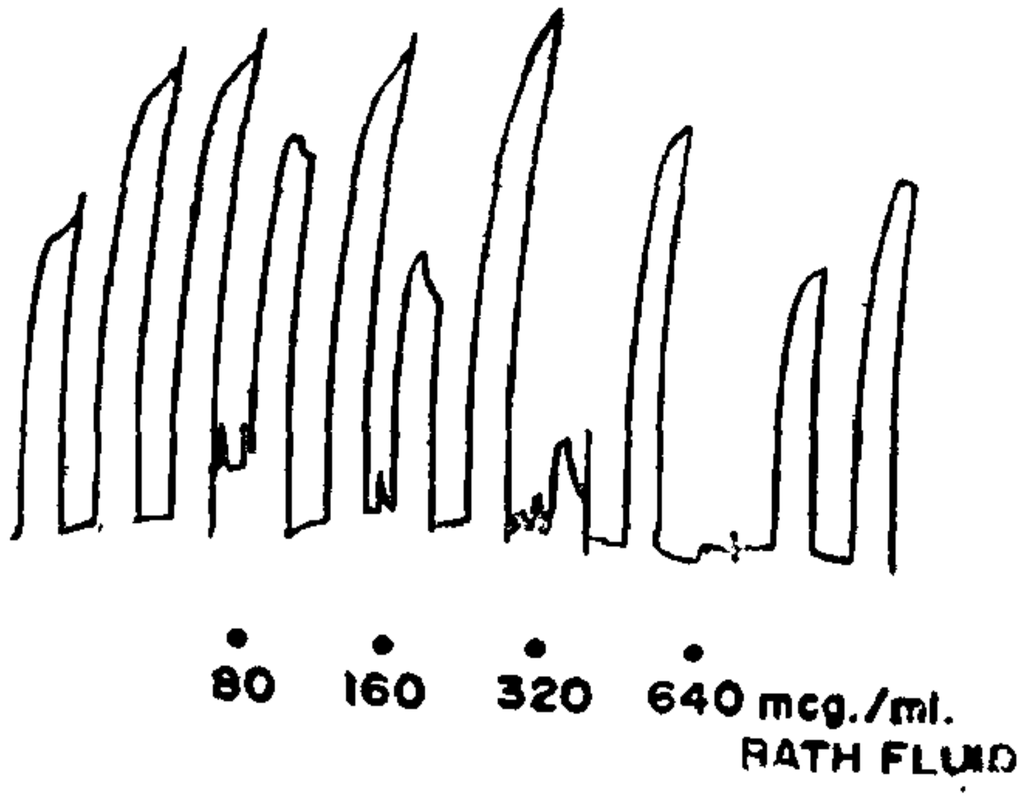


FIG. 1. Isolated guinea pig liuem; Representative record of an experiment showing progressive reduction of acetylcholine responses (0·6μg/ml; time of contact— 45 seconds) by incremental increases in concentrations of compound II (80–640 μg/ml) added 1 minute before acetylcholine administration, Drum speed: 5 mm/min.

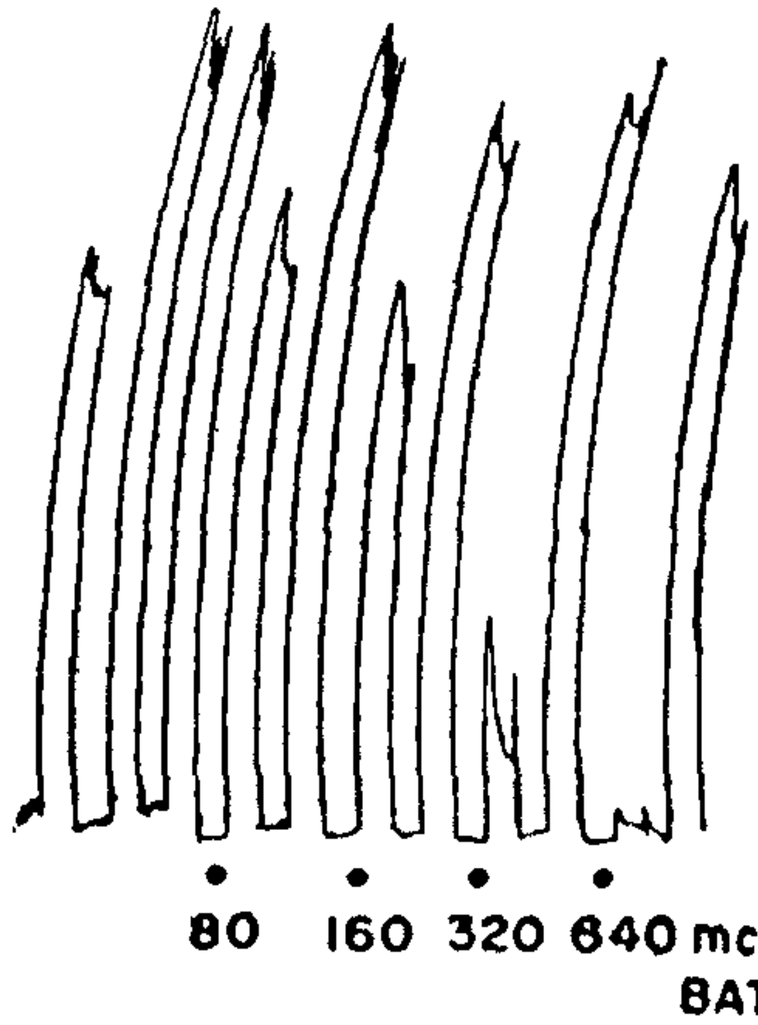


FIG. 2. Isolated guinea pigileum : Representative record of an experiment showing progressive reduction of histamine responses (0·003 μg/ml; time of contact— 30 seconds) by incremental increases in concentrations of compound III (80–640 μg/ml) added 1 minute before histamine administraton. Drum speed: 5 mm/min.

Incremental increases in the concentrations of compounds from 0·25 mg–4·0 mg/ml bath fluid induced a dose-dependent reduction in the frequency of spontaneous contractions. Usually, it was observed that at concentrations above 2 mg/ml bath fluid, the ileum became paralyzed which could be restored only after repeated washings (Fig. 3). It was further observed that the contractile force and frequency of contraction of rabbit jejunum preparations (3 experiments for each compound) increased by pretreatment with acetylchloine (0·01 and 0·02 μg/ml bath fluid) could be reduced by the addition of the compounds (2 or 4 mg/ml bath) indicating that the compounds could interfere with either drug-induced or spontaneously occurring contraction.

In guinea pig tracheal chain experiments (3 experiments for each compound) the agonists used were histamine and acetylcholine. The minimum amount of the compound required to produce relaxation was 0·5 mg/ml of bath fluid. Incremental increases in the concentrations of the compounds (0·5–4 mg/ml bath fluid) produced dose-dependent reduction in the height of acetylcholine or histamine-induced sustained contractions. The behaviour of these compounds was much like that of an antihistaminic or an acetylcholine-blocker drug (Fig. 4).

Cardio-vascular Response to Compounds II and III

In experiments with cats, intravenous injection of the compounds (20 mg/kg) produced a biphasic effect on mean arterial blood pressure. An initial vasodepressor effect which lasted for 5–10 minutes was followed by a secondary pressure response (Figs. 5 and 6). Repeated intravenous administration of these compounds showed no evidence of tachyphylaxis or anaphylaxis, *i.e.*, the vascular response neither decreased nor increased.

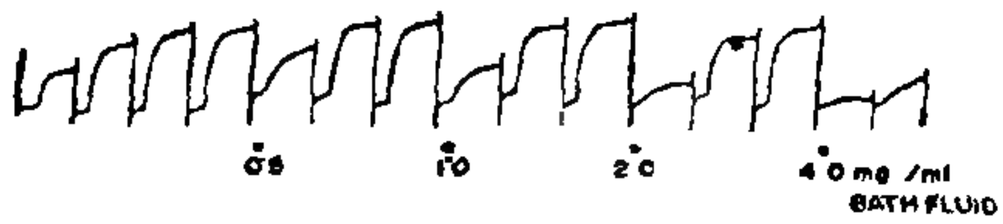


FIG. 4. Isolated guinea pig trachea : Representative record of an experiment showing progressive reduction of acetylcholine response (4 μg/ml; time of contact—1 min. 30 sec.) by incremental increase in concentrations of compound III (0·5–4 mg/ml) added 2 minutes before acetylcholine administration. Drum speed : 5 mm/min.

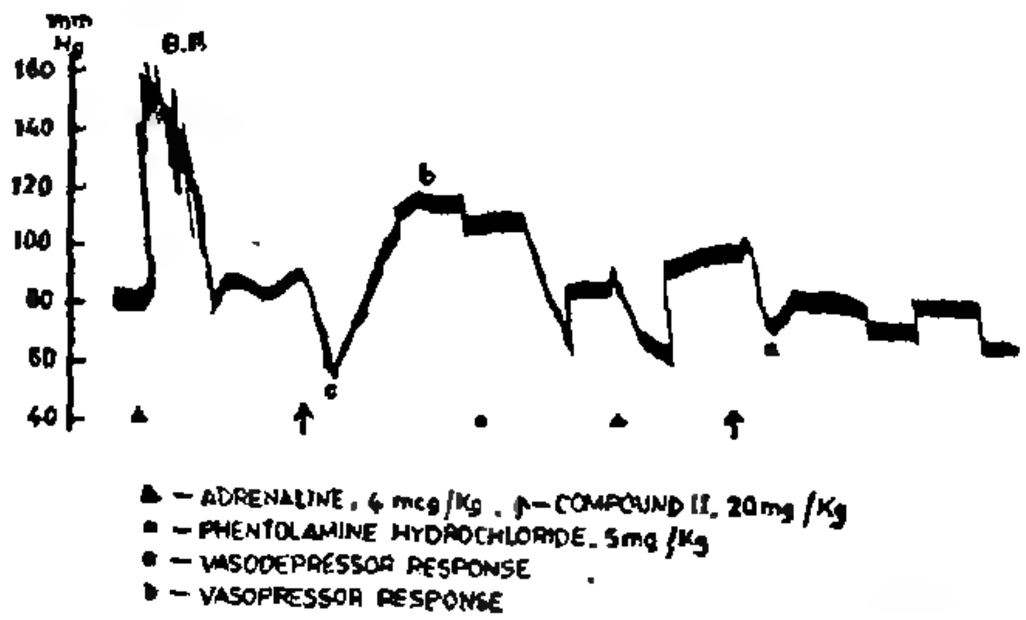


FIG. 5. Representative record of an experiment showing the effects of compound II on anaesthetized cat's blood pressure. The vasodepressor response (*a*) was followed by vasopressor response (*b*). A test dose of adrenaline 4 μg/kg) was totally blocked after 5 hour of phentolamine hydrochloride administration (5 mg/kg i.v.) which eventually could not modify the vasodepressor response (*a*), but checked the vasopressor response (*b*) completely.

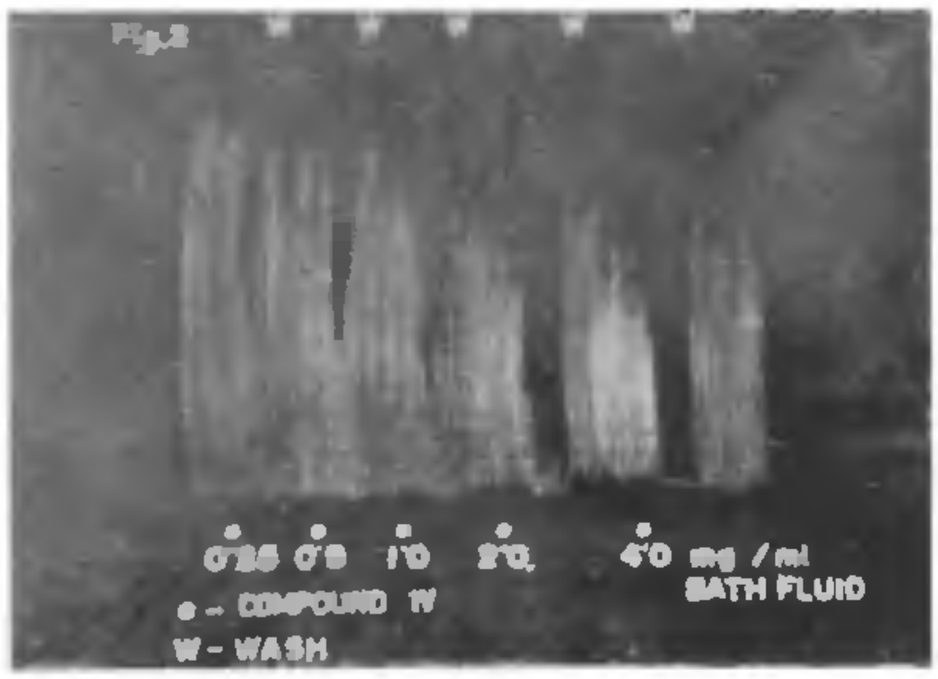


FIG. 3. Isolated rabbit ileum : Representative record of an experiment showing progressive inhibition of normal pendular contractions with incremental increases in concentrations of compound IV 0·25–4 mg/ml). At concentrations above 2 mg/ml of compound IV the ileum was found paralyzed. Normal rhythm could be restored after repeated washings. Drum speed : 5 mm/min.

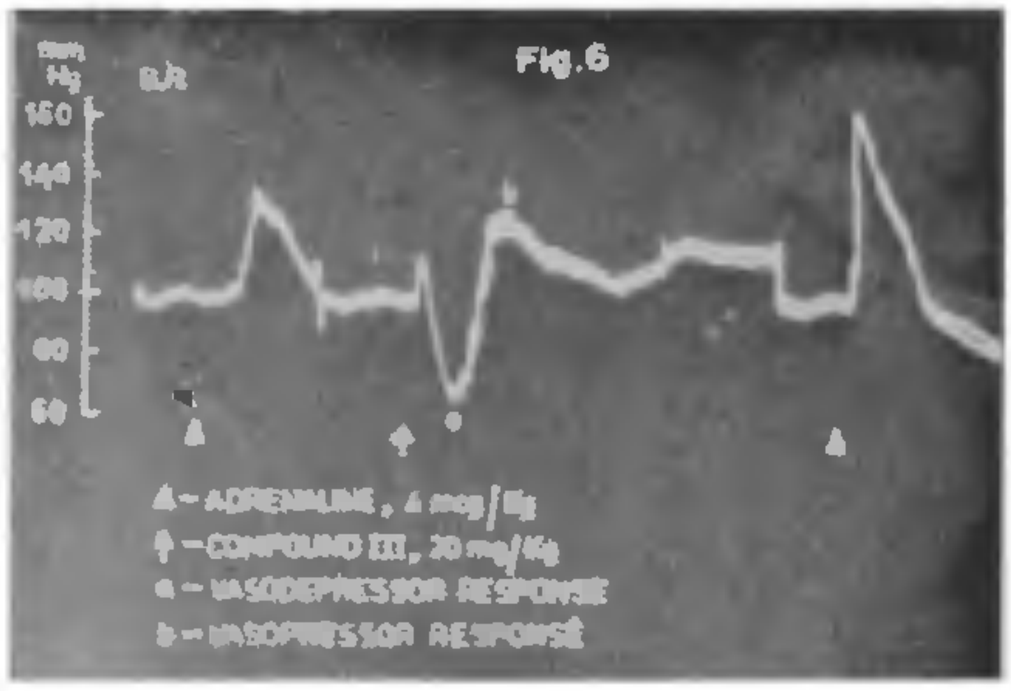


FIG. 6. Representative record of an experiment showing the potentiation of vasopressor response to a test dose of adrenaline (4 μg/kg) after 30 minutes of administration of compound III (20 mg/kg) on anaesthetized cat's blood pressure.

Vasodepressor Response to II and III

To determine if the vasodepressor response to the compounds was due to the release of endogenous histamine, a satisfactory histamine block was produced by the intravenous injection of antazoline methanesulphate, 4 mg/kg for 20 minutes[2]. The intravenous injection of the compounds produced a vasodepressor response in all animals that was identical in pattern and degree to the pre-block control response except in case of compound II the vasodepression was significantly potentiated ($P < 0{\cdot}05$; Table I). To determine if the vasodepressor response to the compounds was due to direct stimulation of β-adrenergic receptors, complete β-receptor block was produced by propranolol hydrochloride, 0·3 mg/kg for 20–30 minutes[2]. Intravenous injection of the compounds produced a vasodepressor response similar to the pre-block control response (Table I). To determine if the componuds produced vasodepressor response by elaboration of mediator acetylcholine, they were administered after producing acetylcholine block with atropine sulphate, 1 mg/kg intravenously for 45 minutes[2]. The vasodepressor response in all animals was identical in pattern and degree to the pre-block control response (Table I). To determine if the compounds produced vasodepression through stimulation of α-adrenergic receptors, they were injected after producing α-receptor blockade with phentolamine hydrochloride, 5 mg/kg for 1 hour[2]. The vasodepression observed was similar in all respects to pre-block control response with the compounds (Fig. 5). Elimination of effect of higher centres by spinal transection or of vagal stimulation by bilateral vagotomy could not modify the vasodepression effect of both the compounds.

Vasopressor Response to II and III

The vasodepression produced by the compounds was followed by a compensatory overshooting rise of blood pressure much higher than the basal blood pressure (Figs. 5 and 6). The response persisted after spinal transection or bilateral vagotomy. After α-receptor block with pentolamine, 5 mg/kg[2] for 1 hr, the compounds behaved differently. The after rise of blood pressure was slightly diminished by III (the change was not statistically significant) and completely checked by II (Fig. 5, Table II). After β-receptor blockae with propronolol, 0·3 mg/kg[2] for 20–30 minutes the vasopressor effect of III was checked but that of II was enhanced ($p < 0{\cdot}05$; Table II).

TABLE I

Vasodepressor actions of ω-amino-p-nitroacetophenone hydrochloride (II) and ω-acetamido-p-nitrophenone (III) in anaesthetized cats : effect of pre-treatment with blocking agents

Blocking agents administered intravenously	Per cent reduction in blood pressure in mm Hg	
	Before administration of blocker	After administration of blocker
Compound II (20 mg/kg, i.v.)		
1. Antazoline methanesulphate (4 mg/kg for 1 hr)	45 ± 2	55 ± 4*
2. Atropine sulphate (1 mg/kg for 45 min.)	37 ± 7	42 ± 6
3. Propranolol hydrochloride (0·3 mg/kg for 20–30 min.)	33 ± 4	44 ± 6
4. Phentolamine hydrochloride (5 mg/kg for 1 hr)	15 ± 1	23 ± 9
Compound III (20 mg/kg, i.v.)		
1. Antazoline methanesulphate (4 mg/kg for 1 hr)	35 ± 8	47 ± 5
2. Atropine sulphate (1 mg/kg for 45 min.)	26 ± 3	36 ± 9
3. Propranolol hydrochloride (0·3 mg/kg for 20–30 min.)	42 ± 5	45 ± 4
4. Phentolamine hydrochloride (5 mg/kg for 1 hr)	48 ± 10	35 ± 5

Values are mean of observations from 5 cats ± S.E. The mean control pressure was 94 ± 8 mm Hg.
* $P < 0{\cdot}05$.

TABLE II

Vasopressor responses of ω-amino-p-nitroacetophenone hydrochloride (II) and ω-acetamido-p-nitrophenone (III) in anaesthetized cats

	Drug/Blocker used	Per cent rise in blood pressure after various treatments	P values compared to respective controls
II, 20 mg/kg, i.v.	No blocker (control)	21 ± 4	
	+ pre-treatment with phentolamine hydrchloride (5 mg/kg) for 1 hr	0	
	+ pre-treatment with propranolol hydrochloride (0·3 mg/kg) for 20–30 min.	34 ± 4	< 0·05
III, 20 mg/kg, i.v.	No blocker (control)	19 ± 7	
	+ pre-treatment with phentolamine hydrochloride (5 mg/kg) for 1 hr	12 ± 6	
	+ pre-treatment with propranolol hydrochloride (0·3 mg/kg) for 20–30 min.	0	
Adrenaline, 4 μg/kg, i.v.	No blocker (control)	54 ± 2	
,, ,,	+ pre-treatment for 1 hr with II (20 mg/kg)	81 ± 3	< 0·001
,, ,,	+ pre-treatment for 1 hr with III (20 mg/kg)	99 ± 2	< 0·001

Values are mean of observations from 5 cats ± S.E.
The mean control blood pressure was 92 ± 1 mm Hg.

Adrenaline injected intravenously in cats pre-treated with II and III produced a potentiated vasopressor response in comparison with the control adrenaline-induced vasopressor response (Fig. 6; Table II). However, on repeated administration of compound II at intervals of 30 minutes tachyphylaxis developed for the secondary vasopressor response. Per cent rise in blood pressure was 103 ± 9 (first dose), 76 ± 7 (second dose), 8 ± 2 (third dose) and 0 (fourth dose). The values are mean of observations from 5 cats ± standard error.

Compound IV

Unlike the compounds II and III, the compound IV did not show any appreciable vasodepression or secondary vasopressor response.

DISCUSSION

The results of the smooth muscles experiments clearly indicated that the compounds not only inhibited the autogeneous contractile mechanism (Fig. 3) but were also active when the contractile drive was mangified by use of agonist. The degree of inhibition was related to the concentrations of the compounds in the bath. The action could be reversed by removing the compounds by washing the organ preparation in all the experiments performed. The results point to a possible involvement of a direct non-specific relaxing effect of these compounds.

The persistent hypotensive effect of compounds II and III after intravenous injection could not be inhibited by mediator blocking agents like antazoline methane sulphate, atropine sulphate, propranolol hydrochloride and phentolamine hydrochloride (Table I). This was sugestive of direct action of the compounds on vascular smooth muscle which led to vasodepression.

The compensatory rise of blood pressure after transient vasodepression observed after injection of compounds II and III could be prevented by *a*-receptor block (compound II, Fig. 5) and *β*-receptor block (compound III). Adrenaline induced vasopressor response was potentiated by these compounds (Table II, Fig. 6). These vascular effects were not mediated through some compensatory reflex mechanism because bilateral vagotomy and spinal transection failed to alter the vascular response. The comound II acted as a substrate for monoamine oxidase like adrenaline and nor-adrenaline and the compound III inhibited rat liver mitochondrial monoamine oxidase[6]. The phenomenon of tachyphylaxis also was observed for the vasopressor effect of compound II after repeated administration. All these events indicated that the secondary rise of blood pressure was a sympathomimetic effect. The vascular effects of compound III were similar to the vascular effects of chloramphenicol and its hydrolytic product[3]. All the three compounds showed monoamine oxidase inhibitor properties[6]. The compound IV although had smooth muscle relaxant

properties and inhibited rat liver mitochondrial monoamine oxidase[6] did not show any appreciable cardiovascular effect. The cardiovascular effects of compounds II, III, V and VI had been, however, similar. It is, therefore, difficult to explain in structure-activity relationship of chloramphenicol and its intermediates.

ACKNOWLEDGEMENTS

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1. Banerjee, S. and Mitra, C., *J. Pharm. Sci.*, 1976, **65**, 704.
2. —, — and Mukherjee, A. K., *Ibid.*, 1977, **66**, 1239.
3. —, Basu, P. S. and Mitra, C., *Ibid.*, 1978, **67**, 480.
4. Evans, R. M., *The Chemistry of the Antibiotics used in Medicine*, Pergamon Press, London, 1965, p. 15.
5. Ghosh, M. N., *Fundamentals of Experimental Pharmacology*, Scientific Book Agency, Calcutta 1971, p. 70.
6. Banerjee, S. and Basu, P. S., *Indian J. Biochem. Biophys.*, 1978, **15**, 479.

INDUCED APOGAMY IN *ADIANTUM TRAPEZIFORME* L.

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ABSTRACT

In vitro grown prothalli of *Adiantum trapeziforme* L. were subjected to various sucrose concentrations. Gametophytic callus initiated from the prothalli produced apogamous shoots in the presence of sucrose. The results obtained showed, that exogenous supply of sugar especially sucrose, plays a key role in generating apogamous response.

INTRODUCTION

INDUCED apogamy has proved to be of great interest because it does not appear to involve any specific genetic change and its occurrence can to a degree at least be brought about under experimental control. From *in vitro* studies, the factor responsible for the induction of apogamy appears to be a high level of carbohydrate (Bopp[1]), as also undoubtedly is the prevention of fertilization because of unsuitable physical or physiological environment (Nair and Kaur[4]).

The present investigation deals with morphological changes that occurred in the *Adiantum* prothalli when subjected to various sucrose levels. Moreover, the morphogenic potentiality of the gametophytic callus was also examined.

MATERIALS AND METHODS

Mature spores of *Adiantum trapeziforme* L. were collected. The spores were sterilized with 5% sodium hypochlorite for 5 minutes. The sterilized spore suspension was inoculated on slants of Knudson's medium, as modified by Steeves *et al.*[5]. Cultures were incubated at 25 ± 2°C in continuous light in a culture room.

RESULTS

Effect of sucrose concentration on prothalli

Four week old prothalli were inoculated on Knudson's medium containing 1%, 2% and 4% sucrose respectively. Few prothalli were inoculated on Knudson's basal medium (sucrose free). After 4 weeks incubation, prothalli grown on 4% sucrose medium became quite thick and developed profuse hair (Fig. 1). The formation of hair and the prothalli becoming quite thick had been observed to be the external indication in apogamous shoot formation. On further incubation, well developed shoots were observed from these prothalli (Fig. 2). By this time, there was no apogamous response from prothalli grown in media containing low sucrose concentration (2% and 1%). Prothalli grown on basal medium remained quite thin.

Initiation of callus on prothalli

Knudson's medium containing 2% sucrose, 10% coconut milk and supplemented with 1·0 mg/l and 2·0 mg/l 2,4-D were inoculated with 2, 4, 6 and 8 weeks old prothalli. After 4 weeks incubation 6 and 8 weeks old prothalli grown on medium containing 2% sucrose, 10% coconut milk and 2·0 mg/l 2,4-D