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Some *in vitro* and *in vivo* Pharmacological Observations on Paraphenylene Diamine (Hair Dye)

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Abstract: Objectives: Paraphenylene diamine (PPD) is commonly used in our region as hair dye, and to itensify Henna color. Several cases of poisoning with this compound have been reported. Therefore, we have examined the actions of PPD on a variety of pharmacological preparations in an attempt to determine the basis of its toxicity. Methods: Several isolated rat, rabbit, frog and Guinea pig preparations were used, together with anesthetized cat for blood pressure measurement. Results: After incubation of PPD with chopped G. Pig lung tissue, the supernatant was found to contract G. Pig illeum. This action was abolished by chlorpheniramine, suggesting that PPD released histamine. PPD, or the chopped lung tissue preparation, given alone, did not contract G. Ileum. Various doses of PPD did not affect striated muscle preparations, nor did it affect isolated tissues preparation, given alone, did not contract *G. Ilenum*. Various doses of PPD was also without effect on prostaglandin syntheis or receptors. No significant change in blood pressure was observed following PPD injection at low doses in anaesthetized cats, whereas high doses were lethal. Conclusion: PPD was ineffective in significantly altering the reactivity of several pharmacological preparations. It suggested the PPD releases histamine.

Key words: Paraphenylene diamine, hair dye, pharmacdynamics

Introduction

Paraphenylene diamine (PPD) is a syntheitc compound that is widely used as a hair dye, in photochemical measurements, and also in manufacturing of tire cords and photographic developer (Macphee and Podger, 1975; Burnett and Corbett, 1977). Several cases of accidental, homicidal, and suicidal poisoning with PPD have been reported in the Sudan, where it is mainly syued to intensify the black color produced by Henna (Lawonia inermis), and to reduce the time required for dying and decorating hands and feet with Henna (El-Ansary *et al*., 1983; Suliman *et al*., 1983; Yagi *et al*., 1991; Abdel Karim *et al*., 1992). PPD has also been identified as one of the constituents of 'home doctors' (Averbukh *et al*., 1989).

As far as we are aware, there is no published work on the basic pharmacology of PPD. Therefore, we assessed its effect son some pharmacological preparations *in vitro* and in *vivo.* Our results may elucidate, at least partially, some of the toxicological actions of the dye.

Materials and Methods

Animals: Rabbits (White New Zealand strain, about 1.5 Kg), cats (local breed, 1.5-2.0 Kg), rats (Wistar strain, 180-200 g), and frogs (40 g) of both sex were used. They were supplied and maintained in the facilities of the Animals Hosue of the Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan). Nutritionally adequqate feed and water were supplied to the animals *ad libitum,* except where mentioned.

***In vitro* experiments (isolated preparations):** All the pharmacological preparations tested in this work were conducted essentially as described by Kitchen (1984). These include: Frog rectus abdominus muscle, Isolated rat uterus, Rat fundus strip, Isolated perfused rabbit heart, Rabbit aortic strip, Rat ascending colon, Guinea (G) pig ileum, Chopped G. Pig lung and Rat phrenic nervehemidiaphragm.

***In vivo* experiments:** Anesthetized at blood pressure (Kitchen, 1984).

Drugs and Chemicals: All chemicals were analytical reagent grade. PPD and serotonin were bought from Sigma (St. Louis, MO, USA).

Statistical analysis: Values reported are means ± SEM (number of observations). Differences between means were estimated by the Student's test, and probability (P) given. P less than 0.05 was considered significant.

Results

In vitro experiments

Frog rectus abodminus: PPD (1-500 μg/ml) had no effect when added alone to this preparation. However pre-addition of PPD (>10 μg/ml) reduced the sensitivity of the tissue to carbachol. At a concentraion of 300-500 μg mL^{-1}, the tissue lost its sensitivity to carbachol (0.5-2.0).

Isolated rat uterus: PPD (100-200 μg/ml) did not produced an effect on this preparation when added alone. Pre-addition of PPD (>500 μg/ml) reduced the sensitivity of the tissue to carbachol (1 μg/ml). At a concentration of 600-2000 μg/ml of PPD, the tissue lost its sensitivity to carbachol (2 μg/ml).

Rat fundus strip: PPD (100-2000 μg/ml) did not affect this preparation when added alone. Pre-addition of PDD (>200 μg/ml) reduced the sensitivity of the tissue to serotonin (5-HT). At 600-2000 concentration of PPD, the tissue lost its sensitivity to 5-HT (0.5-2000 μg).

Isolated perfused rabbit heart: PPD, at concentrations of 500-1000 μg/ml, produced a slight increment in the contractility of the heart.

At concentrations of 5-40 mg/ml, it significantly and dose-dependently increased contractility ($p<0.01$). At a concentration of 40 mg/ml the increased contractility ($p<0.01$). At a concentration of 40 mg/ml the increase in cardiac contractility was followed by an irreversible decrease. The cardiac output tended to decrease with increasing concentrations of PPD. The

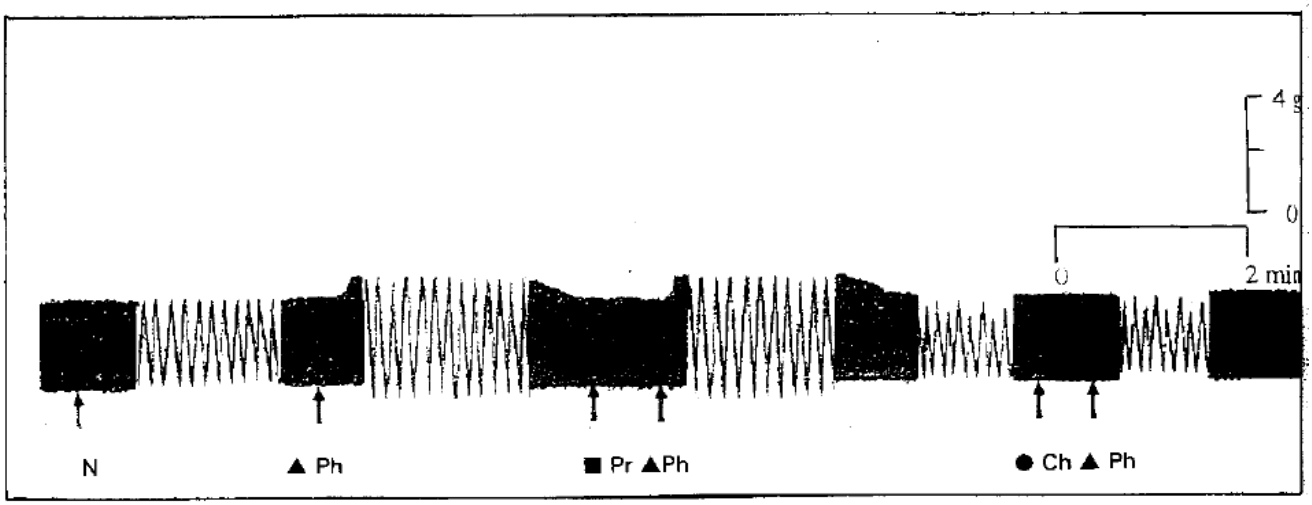

Fig. 1: The effect of PPD on perfused rabbit heart. PPD(▲ Ph 5 mg) increased constractility. Pre-addition of Propranolol (■ Pr 10 μg) did not influence PPD effect. However pre-addition of chloropheniramine (● Ch 10 μg) blocked the effect.

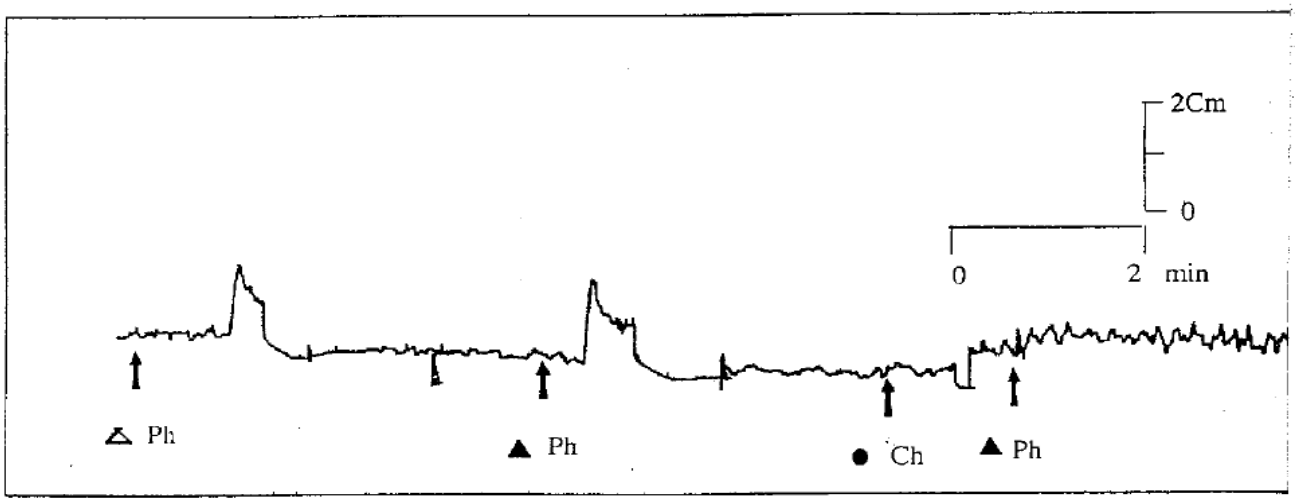

Fig. 2: The effect of PPD lung incubate on guinea - pig ileum. Administration of authentic PPD (▲ Ph 1ml) and comercial PPD lung perfusate (△ Ph 1ml) on guinea - pig ileum produced marked contraction. These effects were blocked by pre-addition of chloropheniramine (● Ch. μG).

Table 1: The effect of paraphenylene diamine (PPD) on perfused rabbit heart.

Dose (mg)	Contractility (g of tension)	Heart rate (beat/min)	Cardiac output (ml/min)
Control	5.57 ± 0.61	121.0 ± 7	6.43 ± 0.2
0.5	5.77 ± 0.61	118.0 ± 7	6.29 ± 0.3
01	6.14 ± 0.67	118.0 ± 10	6.21 ± 0.2
05	7.33 ± 0.70	128.0 ± 12	6.21 ± 0.2
10	7.51 ± 0.71*	137.0 ± 18	6.07 ± 0.2
20	6.99 ± 0.88*	108.0 ± 13**	5.21 ± 0.4**
40	7.24 ± 0.60	81.0 ± 14**	4.67 ± 0.5**

Values are mean ± SEM (n = 6)
*P < 0.05, **P < 0.01 (compared to control

heart rate tended to increase with increasing doses of PPD (5-10 mg/ml). At concentrations of 20-40 mg/ml, however, the heart rate significantly decreased (P < 0.01).

Pre-addition of propranolol (10 μg) did not alter the increases in contractility and cardiac output that were produced by PPD (5 mg/ml). Pre-addition of chlorpheniramine (10 μg) abolished the effects produced by PPD, while the pre-addition of propranolol was ineffective (Fig. 1).

Rabbit aortic strip: The addition of PPD (4-1000 μg/ml) did not affect this preparation, nor did it alter the sensitivity of the tissue to noradrenaline (2 μg mL^{-1}).

Rat ascending colon: PPD (100-3000 mg/ml) did not produce significant effects when added alone to the preparation. Pre-addition of PPD (9-200 μg/ml) reduced the sensitivity of the tissue to carbachol (0.5 μg/ml). At a concentration of 3 mg/ml PPD, the tissue became insensitive to carbachol (0.5 μg/ml).
Chopped G. Pig lung and G. Pig ileum: Chooped lung tissues co-incubated with PPD (2 μg/ml) contracted the ileum. Pre-addition of chlorpheniramine (2μg/ml) to the gut bath abolished the contraction (Fig. 2).

Rat phrenic nerve-hemidiaphragm: PPD (10-400 μg/ml) did not affect this tissue. At higher concentrations (>500 μg/ml), PPD irreversibly inhibited the electrically-stimulated muscle twitches.

***In vivo* experiments**
Cat blood pressure: Intravenous (IV) injection of PPD (2-71.5 μg/Kg) did not exert any significant action on blood pressure of anaesthetized cats. However, higher doses (>71.5 μg/Kg) were lethal to the animals.

Discussion

The present results indicate that PPD doses not affect straited muscles, since treatment with low concentrations of this substance did not produced agonistic or antagonistic effects on frog rectus abdominus muscle or diaphragm. However, higher concentrations caused irreversible reduction or loss of tissue sensitivity to carbachol or to electrical impulses. This may be related to the nectotic action of PPD on muscles, nerves, or both (Yabe, 1992). PPD, likewise, did not affect other preparations that are rich in typtaminergic, adrenergic or muscarinic receptors, indicating the lack of activity at these receptors. Previously, PPD was tested on skinned muscles, and it was postulated that PPD might cause leakage of Ca^{+2} from the sarcoplasmic reticulum, which consequently causes changes such as continuous contractions, that may lead finall to irreversible damage (Yabe, 1992).
The rat ascending colon did not respond to PPD. The activity of this preparation is known to be mediated via prostaglandins (PG) (Vane, 1971). This indicates that PPD has neither PG-like action, nor the ability to alter the synthesis of endogenous PG. Similarly, the lack of effect of the dye on the colon may indicate that it does not affect the autonomic innervation of the preparation (Table 1).
PPD produced a significant positive inotropic and chronotropic effect on the ioslated perfused rabbit heart. This effect cannot be attributed to beta receptors stimulation, since it was notblocked by the pre-addition of propranolol. The effect of PPD on the heart was blocked by chlorpheniramine. This effect may be due to the stimulation of H_1 and H_2 receptors of the heart, which have inotropic and chronotropic effects (Douglas, 1985). Cardiac muscles continuously produce histamine, and the isolated heart is considered a suitable preparation for the study fo histamine release (Giotti *et al*., 1966). It is possible that PPD releases histamine from mast cells.
G. Pig ileum was clearly contracted by a supernatant of G. Pig lung tissue that had been incubated with PPD. PPD alone (withour lung tissue) caused no contraction. This effect was probably mediated by H_1 receptors, because it was blocked by chlorpheniramine. This experiment confirmed the histamine-releasing property of PPD. G. Pig lung was used here because of its high mast cells content, and G. Pig ileum because of the presence of H_1 receptors (Kitchen, 1984). The mechanism by which PPD releases histamine is not certain. It may involve impairment of the cellular events linked to exocytosis (Hazama *et al*., 1992). Disturbances in cell membrnces leading to cell lysis (Lau and Pearce, 1990), or to opening of certain Ca ion channels leading to elevated intracellular Ca^{2+} concentration, which, in turn, may activate mast cell secretion (Eleno *et al*., 1990). The histamine-releasing property of PPD may provide an explanation for the decresae in the rate and force of rabbit isolated heart, since histamine is known to have direct actions on the heart, by promoting Ca^{2+} influx and hastening diastolic depolarizatin in the sinoaterial node. It also slows atrioventricular (AV) conduction to increase automaticity and elicit diverse arrhythmia (Douglas, 1985). These actions are ascribed to H_2 receptors, except for the AV conduction, which is attributed to H_1 receptors (Douglas, 1985). As the contractility of the isolated heart was blocked by an H_1 receptor antagonist, it may be that there is a loss of selectivity at higher doses, or that there is a species difference.
PPD, as a histamine releaser, may be without effect on the autonomic nervous system or the neuromuscular junctino. Our contention that PPD is a histamine-releaser, is supported by the observation that PPD exposure has been associated with increased histamine tissue levels, hypersensitivity and allergic reactions in G. Pigs (Mathur *et al*., 1990). It has also been shown that PPD induces contact dermatitis by causing oxidative stress in the keratinocytes (Picardo *et al*., 1991), whereas Rajaka and Blohm (1970) suggested that benzoquinone formation plays an important role in the allergic reaction to PPD. Ng-Sk, in singapore, considered PPD as one of the chemicals that can produced a different type of contact dermatitis (Ng, 1990). It was also found that challenging humans with PPD produces a positive reaction (Zhao and Fan, 1991).
This study has shown that PPD was ineffective in altering the reactivity of several pharmacological preparations. However, it apparently is a histamine releaser, and this may explain its toxicity in chicks and rats.

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