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THE EFFECTS OF ACETYLSALICYLIC ACID ON THE PITUITARY PROLACTIN OF THE LIZARD, *UROMASTIX HARDWICKII*

MAHMOOD AHMAD, RUQAIYA HASAN, MANSOOR AHMAD*,ANILA QURESHI* AND SHAMA MASROOR

Department of Physiology, University of Karachi, Karachi-75270, Pakistan
**Department of Physiology, New York Medical College, and Westchester Hospital, Valhalla, New York, U.S.A.*

This study deals with the intravenous administration of 7mg acetylsalicylic acid (ASA) solution to *Uromastix hardwickii* for 4 days. It enhances the activity of anterior pituitary lactotrophs, when 0.1 ml of pituitary homogenate of ASA treated was injected hypodermically to crop- sac showed a greater diametric response and increased activity with milk like secretion than that of the injections of 0.1 ml homogenate of control pituitary. The present study indicated that ASA induces hyperprolactinemia.

Keywords: Acetylsalicylic acid, Uromastix pituitary, lactotrophs

INTRODUCTION

Aspirin (acetylsalicylic acid, ASA) is the best known and extensively prescribed salicylate. It is an analgesic, antipyretic and anti-inflammatory agent belongs to non steroid anti-inflammatory drug class (NSAIDs) (Kacso and Terezhalmy, 1994). In larger doses ASA produce measurable anti-inflammatory changes in patients suffering from rheumatoid arthritis (Boardman and Hart , 1967; Deodhar *et al*., 1973 and Botting, 1999) ASA is the classical anti thrombotic agent and is widely used for its anti platelet activity (Mueller, 2004 and Niv *et al*., 2005).

NSAIDs have multiple pharmacological effects with multiple mechanisms of actions. In both, animals and man large doses of Salicylates cause hyperglycemia and glycosuria, through depletion of liver and muscle glycogen (Randall, 1963; Mitzkat and Schonhofer, 1978). High doses of salicylates activate central sympathetic centers and cause release of epinephrine from the adrenal medulla; though this release is partially responsible for the hyperglycemia and depletion of liver glycogen. On the other hand steroid secretion is stimulated by adrenal cortex through their effect on the hypothalamus and increase transiently the plasma concentrations of free adrenocorticoids by displacement from plasma proteins (Paulus and Whitehouse, 1973).

ASA exhibits many side effects and manifest the toxic actions on gastric mucosa and kidney. Chronic use of ASA in managing inflammatory disorders are associated with a high prevalence of gastric and duodenal ulceration (Smith *et al*., 2000 and Niv *et al*., 2005).

ASA directly or indirectly influences the endocrine system. It reduces the plasma levels of thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) in human (Langer *et al*., 1978). In mammals it elevates prolactin (PRL) levels (Luigi *et al*., 2001 and Ahmad *et al*., 2005) and reduces cortisol response (Cavagnini *et al*., 1979).

In the present investigation ASA was administered intravenously to *Uromastix hardwickii* and its role to initiate prolactin secretion through hypophyseal system was determined by a crop-sac receptors response.

MATERIALS AND METHODS

Assay animals

In all twenty four *Uromastix hardwickii* were used in this study to investigate the effects of acetylsalicylic acid on pituitaries. The lizards were obtained from local suppliers and were kept in the laboratory at a constant room temperature of 29+1^{o}C (Ahmad and Taqawi, 1978a, b, 1979 and Ahmad *et al*., 1980).

Twenty four pigeons aging 8-10 week, belonging to white race were used for crop-sac assay. The birds were obtained from local breeders and housed in the laboratory for 5 days; keeping one pigeon in one cage. They were fed with millet and water ad libidum (Ahmad *et al*., 2001a, b and 2002a).

Drug information

Aspirin was a name first used in the late nineteenth century for acetylsalicylic acid (ASA). The first pharmacological data on aspirin appeared in 1899. At present, aspirin is probably used more widely and in larger quantity than any other therapeutic agent. Salicylates are most commonly used as antipyretic and analgesic agent in the relief of headaches, of minor aches and pains. But the most important use is in the treatment of arthritis and rheumatic fever (Donald, 1992).

The various preparations related to salicylic acid are referred to as salicylates. The salicylates are rapidly and completely absorbed from the stomach and upper small intestine, providing a more acidic medium in the stomach. After its absorption, salicylates are rapidly distributed throughout the body tissues and most transcellular fluids primarily by pH dependent passive processes. The drug rapidly crosses the placental barrier. It is not secreted in the gastric juice. Only tracer amounts of salicylates are present in bile, sweat and feces. ASA is absorbed as such and hydrolyzed to acetate and salicylate by esterases in blood (Walson and Mortensen, 1989). The important action of ASA in relieving pain and reducing inflammation is most probably due to an action at the site of origin of the pain rather than an alteration of perception of the pain by an action on the CNS. Reduction of inflammation and pain is probably due to vasodilatation (Durand *et al*., 2002).

ASA is a comparatively strong acid, having a carboxyl group with a pKa of 3.5 that is in a solution with pH 3.5, half as the ionized salt form. Therefore, administration of ASA in combination with an alkali would decrease the rate of absorption of the drug from the stomach by increasing the fraction of ASA present as the charged, poorly absorbed salt. Adequately buffered preparations would reduce gastric irritation, and dosimetry is not important (Verbeeck, 1990).

Dosimetry
1 ml of 7 mg ASA solution per day was administered intravenously to each lizard for 4 days. The animals were decapitated on day 5.

Drug administration
In experiment I, a dose of 1 ml aqueous vehicle was administered intravenously to each lizard of a batch of 12 controls. For this purpose a median slit of about an inch was made in the abdominal integument; the incised skin was detached and stretched from the muscles on both the sides to make the abdominal vein visible for intravenous injection through a canula. The incision was later protected with a piece of adhesive plaster. In experiment II a dose of 1 ml of 7 mg ASA solution was given to each lizard. The method of injection adopted was the same; and all the lizards were killed following ASA administration on day 5. No sooner their pituitaries were extracted, suspensions were prepared, tagged and refrigerated.

Removal of lizard pituitaries
The lizards were decapitated on day 5 without using any anesthetic. Their skulls were cleaned of skin and muscles; and the vaults (skull cap) were removed with the help of a pair of large scissors. The brain was scooped out of each skull and the pituitary glands extracted from sella turcica. Each of the pituitaries extracted were tagged for the preparation of suspension for a separate bioassay and refrigerated (Ahmad *et al*., 2002a).

Pituitary suspensions
Suspension of each pituitary was prepared separately by grinding with an agate and a mortar in a small fixed quantity of pyrogen free distilled water. The amount for suspension was 0.4 ml inclusive of pituitary. It was transferred by a hypodermic syringe to a tagged serum bottle and refrigerated till its use for bioassay.

Assay procedure
The procedure adopted for bioassay was that of Ahmad *et al*. (2002b). Twelve pigeons were used in each experiment. Feathers overlying the crop were plucked off six hour before starting the intradermal injections.

In experiment I, 0.1 ml vehicle treated control pituitary suspension of a corresponding tag was injected intradermally in the geometrical centre of each half of the crop-sac. Whereas, in experiment II, 0.1 ml suspension belonging to ASA treated pituitary was injected intradermally in the centre of the right and left half of the crop for 4 days according to the operational scheme.

Diametric measurement
The birds were killed on day 5. The skin was separated, before bisecting the crop sac. The lining of each half was rinsed with tap water and all the adipose tissue was removed. Each half of the crop was then stretched against the light of a table lamp, fitted with 100 watt bulb; since all the tissue is comprised of prolactin receptors; lying closely side by side in a plain. Therefore, a plainmetric measurement of this proliferated circularly opaque area was done thrice with the help of a caliper.

RESULTS

It is necessary to mention that diametric response of less than 2.0 cm and more than 3.85 cm are unreliable to rate (Ahmad *et al*., 2002b). Table 1 indicates that vehicle administered control pituitary suspensions gave a minimum diametric response of 2.06 cm; a maximum of 2.16 cm and a mean crop-sac diametric response of 2.12 ± 0.03 cm. On the other hand, lacertilian pituitary suspensions of ASA treated subjects gave higher diametric response. In tests; a minimum diametric response was 2.35 cm; a maximum of 2.39 cm and a mean of 2.37 ± 0.01 cm (Table 2). Statistical analysis showed that mean crop-sac diameters of control and test groups differ significantly with each other ($p < 0.05$).

DISCUSSION

At the usual therapeutic doses of ASA the main adverse effect is gastric upset, resulting in gastrointestinal ulceration and impaired kidney functions (Niv *et al*., 2005). When larger doses are employed in a few of the most sensitive

subjects other symptoms of salicylism may appear (Henry and Volans, 1984).

The most of the therapeutic uses of ASA are explained through the inhibition of prostaglandins (PGs) synthesis (Tanasescu *et al*., 2000). Arachidonic acid released from membrane phospholipids stores (Fitzpatrick and Soberman, 2001) and converted to PGs by cyclooxygenase (COX).

ASA irreversibly blocks COX pathway by acetylating the COX enzymes (Smith, 1989; Durand *et al*., 2002). This indicates that a single pharmacological effect, that inhibition of COX produces both the therapeutic and side effects of ASA (Botting, 1999). PGs influence the physiological processes by enhancing the release of hormones and/or altering the sensitivity of target organs (Lands, 1991; Mustafa and Srivastava, 1989). Endogenous PGs functional role for stimulation of hypothalamus or pituitary gland has been demonstrated by the use of ASA as inhibitors of their synthesis (Fujimoto, 1978).

Table 1
Crop-sac diametric response following hypodermal injections of 0.1 ml of homogenate of vehicle treated pituitary suspensions

Pigeons No.	Pigeons' weight (g)	*Crop-sac diametric response (cm)
1.	343	2.11
2.	345	2.15
3.	330	2.06
4.	342	2.14
5.	350	2.12
6.	338	2.13
7.	354	2.16
8..	351	2.09
9.	332	2.14
10.	339	2.15
11.	345	2.13
12.	350	2.08
Mean	343.25 ± 7.52	**2.12 ± 0.03

*Each figure is the mean of right and left crop-sac diametric measurements.
**The mean of 24 diametric measurements with ± SD.

Dussault *et al*. in 1976 indicated a significant decrease of TSH concentration after ASA treatment in human but has no effect on PRL concentration suggesting no direct effect of PGs on the release of PRL. Another studies in mice proposed a possible role of PGs as mediators for the actions of PRL in mammary gland (Rillema, 1976). After the administration of ASA in fish PRL levels were increased (Van Anholt *et al*., 2003) suggested that mammalian PGs promote PRL release by decreasing PRL release inhibitory factor and increasing the PRL releasing factor from the hypothalamus without having a direct effect on pituitary (Luigi *et al*., 2001).

Present data indicates that PRL levels were not reduced when COX pathway was blocked, instead ASA accelerates PRL secretion in more than normal amounts that is clearly evidenced when control diametric response is compared with salicylate treated pituitary response. It might be that PRL release is under inhibitory control by PGs, or arachidonic acid itself has a stimulatory effect on the release of PRL.

Table 2
Crop-sac diametric response following hypodermal injections of 0.1 ml of homogenate of ASA treated pituitaries

Pigeons No.	Pigeons' weight (g)	*Diametric response (cm)
1.	345	2.37
2.	352	2.38
3.	350	2.36
4.	338	2.37
5.	330	2.37
6.	346	2.36
7.	336	2.38
8..	351	2.37
9.	341	2.35
10.	334	2.36
11.	332	2.38
12.	340	2.39
Mean	341 ± 7.55	**2.37 ± 0.01

*Each figure is the mean of right and left crop-sac diametric measurements.
**The mean of 24 diametric measurements with ±SD.

REFERENCES

Ahmad M, Adeeba S and Humera A (1980). The effects of hypothalamic lesion on the thrombocytes of the lizard, *Uromastix hardwickii. Z. Mikrosk. Anart. Forsch*., **94**: 2, S. 345-352.

Ahmad M and Taqawi IH (1978a). Radiation induced leukemia and leucopenia in the lizard, *Uromastix hardwickii. Radiobiol. Radiother.*, **19**: 353-360.

Ahmad M and Taqawi IH (1978b). Effect of gamma-rays on the life-shortening of the lizard, *Uromastix hardwickii. Radiobiol. Radiother.,* **19**: 606-611.

Ahmad M and Taqawi IH (1979). The clinical sequence of haematological effects in the lizard, *Uromastix hardwickii* subjected to gamma radiation. *Radiobiol. Radiother.,* **20**: 769-776.

Ahmad M, Mahmood I, Hasan R, Naim T, Fatima H and Ahmad M (2001a). *In vitro* effects of ACTH on pigeon crop-sac epithelium. *Pak. J. Pharm. Sci.*, **14**(1): 19-23.

Ahmad M, Mahmood I, Hasan R, Fatima H and Ahmad M (2001b). Effects of dexamethasone on pituitary prolactin in the lizard *Uromastix hardwickii. Pak. J. Pharm. Sci.*, **14**(2): 43-46.

Ahmad M, Mahmood I, Hasan R, Naim T, Javaid A, Fatima H and Ahmad M (2002a). Bioassay of pituitary prolactin of the lizard *Uromastix hardwickii. Pak. J. Pharmacol.,* **19**(2): 7-11.

Ahmad M, Mahmood I, Hasan R, Fatima H, Javaid A, Naim T and Ahmad M (2002b). Hypoprolactinemia following intradermal administration of ouabain in *Uromastix hardwickii. Pak. J. Pharm. Sci.,* **15**(2): 37-49.

Ahmad M, Hasan R, Ahmad M, Qureshi A, Ahmed Z and Masroor S (2005). The mode of action of acetylsalicylic acid on pigeon crop-sac prolactin receptors. *J. Basic.App. Sci.* (In press).

Boardman L and Hart ED (1967). Clinical measurement of the anti-inflammatory effects of salicylates in rheumatoid arthritis. *Br. Med. J.,* **4**: 264-268.

Botting JH (1999). Nonsteroidal anti-inflammatory agents. *Drugs Today* (Barc), **35**(4-5): 225-235.

Cavagnini F, Di Landro A, Maraschini C, Invitti C and Pinto ML (1979). Effect of two prostaglandin synthesis inhibitors, indomethacin and acetylesalicylic acid, on plasma ACTH and cortisol levels in man. *Acta. Endocrinol.,* **91**: 666-673.

Deodhar SD, Dick WG, Hodgkinson R and Buchanan WW (1973). Measurement of clinical response to anti-inflammatory drung therapy in rheumatoid arthritis. *Q. J. Med.*, **42**: 387-401.

Durand S, Fromy B, Koitka A, Tartas M, Saumet JL and Abraham P (2002). Oral single high-dose aspirin results in a long lived inhibition of anodal current-induced vasodilation. *Br. J. Pharmacol.,* **137**: 384-390.

Donald GP (1992). Nonsteroidal anti inflammatory drugs; nonopioid analgesics; drugs used in gout. *In:* Basic and clinical pharmacology (Katzung, B. G. ed.) 5th ed., pp.491-496. Appleton and Lange, Prentice Hall, U.S.A.

Dussault JH, Turcotte R and Guyda H (1976). The effect of acetylsalicylic acid on TSH and PRL secretion after TRH stimulation in the human. *J. Clin. Endocrinol. Metab.,* **43**(1): 232-235.

Fitz Patrick FA and Soberman R (2001). Regulated formation of eicosanoids. *J. Clin. Invest*., **107**: 1347-1351.

Fujimoto S (1978). Possible roles of prostaglandins in the hypothalamus. *Nippon. Yakurigaku. Zasshi*., **74**(2): 165-177.

Henry J and Volans G (1984). ABC of poisoning. Analgesic poisoning: I salicylates. *Br. Med. J.*, **289**: 820-827.

Kacso G and Terezhalmy GT (1994). Acetylsalicylic acid and acetaminophen. *Dent. Clin. North. Am*., **38**(4): 633-644.

Lands WEM (1991). Biosynthesis of prostaglandins. *Ann. Rev. Nutr*., **11**: 41-60.

Langer P, Földes O, Michajlovskij N, Jezová D, Klimes I, Michalko J and Závada M (1978). Short-term effect of acetylsalicylic acid analogue on pituitary-thyroid axis and plasma cortisol level in healthy human volunteers. *Acta. Endocrinol.* **88**: 698-702.

Luigi DL, Laura G, Francesco R, Carlo B and Domenico C (2001). Acetylsalicylic acid inhibits the pituitary response to exercise related stress in humans. *Med. Sci. Sports. Exerc.,* **12**: 2029-2035.

Mitzkat HJ and Schonhofer PS (1978). Influence of drugs used in obstetrics on carbohydrate metabolism. *Z. Geburt. Perinatol.*, **182**(5): 331-342.

Mueller RL (2004). First-generation agents: Aspirin, heparin and coumarins. *Best. Pract. Res. Clin. Haematol*., **17**(1): 23-53.

Mustafa T and Srivastava KC (1989). Prostaglandins (Eicosanoids) and their role in ectothermic organisms. *Adv. Comp. Environ. Physol*., **5**: 157-207.

Niv Y, Battler A, Abuksis G, Gal E, Sapoznikov B and Vilkin A (2005). Endoscopy in a symptotic minidose aspirin consumers. *Dig. Dis. Sci.,* **50**(1): 78-80.

Paulus HE and Whitehouse MW (1973). Nonsteroid anti inflammatory agents. *Ann. Rev. Pharmacol.,* **13**: 107-125.

Randall LO (1963). Non-narcotic analgesics. *In:* Physiological pharmacology. Vol.1, The Nervous system-part. A. Central Nervous system Drugs. (Root WS and Hofmann FG eds.), Academic Press Inc., New York, pp.313-416.

Rillema JA (1976). Effects of prostaglandins on RNA and Casein synthesis in mammary gland explants of mice. *Endocrinol*., **99**(2): 490-495.

Smith WL (1989). The eicosanoids and their biochemical mechanisms of action. *Biochem. J.,* **259**: 315-324.

Smith WL. De Witt DL and Garavito RM (2000). Cyclooxygenase : Structural, Cellular and molecular biology. *Ann. Rev. Biochem*., **69**: 145-182.

Tanasescu S, Levesque H and Thuillez C (2000). Pharmacology of aspirin. *Rev. Med. Interne. Suppl*., **1**: 18-26.

Van Anholt RD, Spanings T, Koven W and Bonga SE (2003). Effects of acetylsalicylic acid treatment on thyroid hormones, prolactins and the stress response of tilapia (Oreochromis mossambicus). *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **285**: 1098-1106.

Verbeeck RK (1990). Pharmacokinetic drug interactions with nonsteroidal anti-inflammatory drugs. *Clin. Pharmacokin.,* **19**: 44-53.

Walson PD and Mortensen ME (1989). Pharmacokinetics of common analgesics, anti-inflammatories and anti-pyretics in children. *Clin. Pharmacokin.*, **17**(Suppl. 1): 116-120.