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Anticoagulatory Role of an Ergot Mesylate: Hydergine

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***Abstract*—**Thrombosis can be life threatening, necessitating therefore its instant treatment. Hydergine, a nootropic agent is used as a cognition enhancer in stroke patients but relatively little is known about its anti-thrombolytic effect. To investigate this aspect, *in vivo* and *ex vivo* experiments were designed and conducted. Three groups of rats were injected 1.5mg, 3.0mg and 4.5mg hydergine intraperitonealy with and without prior exposure to fresh plasma. Positive and negative controls were run in parallel. Animals were sacrificed after 1.5hrs and BT, CT, PT, INR, APTT, plasma calcium levels were estimated. For *ex vivo* analyses, each 1ml blood aspirated was exposed to 0.1mg, 0.2mg, 0.3mg dose of hydergine with parallel controls. Parameters analyzed were as above. Statistical analysis was through one-way ANOVA. Dunken's and Tukey's tests provided intra-group variance. BT, CT, PT, INR and APTT increased while calcium levels dropped significantly ($P<0.05$). *Ex vivo*, CT, PT and APTT were elevated while plasma calcium levels lowered significantly ($P<0.05$). Our study suggests that hydergine may act as a thrombolytic agent but warrants further studies to elucidate this role of ergot mesylates.

***Keywords*—**Hydergine; Coagulation assays; plasma calcium; ergot mesylates; thrombosis

I. INTRODUCTION

NOOTROPICS like Ginseng, *Ginko biloba* and piracetam that are among the oldest known herbal medicines enhance memory at one end and on the other, decrease viscosity of blood [1]. Hydergine which is also known as dihydroergotoxine or ergot mesylate is one of the well-identified nootropics. It is a peptide alkaloid and consists of an equiproportional mixture of the hydrogenated ergot alkaloids: dihydroergocornine mesylate, dihydroergocristine mesylate and dihydroergokryptine mesylate. Although the metabolic fate of hydergine is not fully known, it is partly absorbed from the gastrointestinal tract and undergoes the first part of metabolism in the liver and less than fifty percent of the dose reaches systemic circulation unchanged. Its elimination from the blood is biphasic with half-lives of 1.5-4 hrs and 13-15 hrs [2, 3]. Hydergine increases blood supply to the brain and inhibits free radical activity; normalizes systolic blood pressure; lowers abnormally high cholesterol levels in some cases; reduces symptoms of tiredness, dizziness and tinnitus [4]. It has proved to be a safe drug during both long and short-term exposure [5].

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Effects of hydergine on local platelet accumulation in the carotid artery as studied by means of the platelet uptake ratio and the systemic platelet vascular wall interactions calculated from platelet half-life have indicated that hydergine decreases *in vivo* platelet residence time to atherosclerotic lesions of the carotid artery suggesting that it could be used for prevention of transient ischemic attacks and atherosclerosis [6].

Moreover, platelet function parameters, such as thromboxane B2 (TXB2), β-thromboglobulin, platelet factor 4, malonalaldehyde (MDA) and ADP-induced aggregation in a placebo study in humans have shown that hydergine treatment decreases platelet activity to a greater extent and improves interaction with the walls of blood vessels to a significant degree [7].

Although evidence exists as regards anti-platelet activity of hydergine but equivalent effects *ex vivo* are not known. Since the previous studies were carried out on humans as placebo trials, hence its anticoagulatory effects after introduction of fresh plasma and dose dependency are unidentified. The present study was attempted in laboratory rats as a model system to examine further the thrombolytic effects of hydergine at different dose regimens under both *in vivo* and *ex vivo* conditions.

II. MATERIALS AND METHODS

A. *Animals and Maintenance*

Experimental procedures regarding animal handling and killing were approved by the local ethics committee on humane use of animals for biomedical research, Department of Animal Sciences, Quaid-i-Azam University, Islamabad Pakistan. Adequate measures were taken to minimize suffering and pain while sacrificing the animals. All animal were fed on rat chow and drinking water *ad libitum*.

B. Selection of the Drug and Dose regimen

For *in vivo* experiments, adult Sprague-Dawley rats (mean body weight = 250 g) were divided into groups of n=5 animals each. Separate groups were injected intraperitonealy with 1.5 mg, 3.0 mg and 4.5 mg hydergine obtained from Novartis Pharma (Pakistan) Ltd. as 3 mg tablets or 0.3 mg/ml ampules while negative and positive controls were treated with 1ml normal saline and 71.4 IU/kg heparin respectively. In another set of experiments, procoagulant was administered in the form of fresh plasma (prepared from the blood of healthy rats) prior to hydergine exposure. Three parallel controls were run; fresh plasma only, fresh plasma and normal saline, and fresh plasma with heparin (71.4 IU/kg).

C. Parameters Studied

Clotting (CT) and bleeding times (BT) of all animals were estimated before and 1.5 hrs after drug administration following standard hematological techniques. Animals were sacrificed 1.5 hrs post-drug administration with an overdose of sodium pentobarbitone (50 mg/$kg_{b.w.}$) and the aspirated blood collected in 3.2% sodium citrate (0.1 ml/0.9 ml blood). It was centrifuged immediately at 1258*g* for 10 min (Eppendorf centrifuge 5810R) to prepare plasma for the determination of prothrombin time (PT), activated partial thromboplastin time (APTT) and calcium levels. PT was estimated with commercially available reagent kit (Sigma Diagnostics Thrombomax®, UK) containing rabbit brain extract with calcium chloride and was expressed in terms of international normalized ratio (INR) calculated using the formula: INR= PT of treated rats ÷ Mean PT of the control rats.

APTT was estimated using a reagent kit (Sigma Diagnostics Alexin^{TM} UK) having rabbit brain cephalin in elagic acid. Both PT and APTT were calculated with a mechanical assay method. Calcium was estimated on a spectrophotometer at 635 nm wavelength using calcium reagent kit (Dia sys Diagnostic System, Germany).

For *ex vivo* experiments, n=5 rats were sacrificed. Blood was aspirated from each rat and to each milliliter of blood 0.1 mg, 0.2 mg and 0.3 mg hydergine was added separately. Corresponding control samples were prepared by adding 1 ml blood to different tubes containing 1 IU heparin, 1 ml normal saline, 1 ml fresh plasma and an empty tube containing pure blood only and no additive for the estimation of CT. Blood taken from another group of rats was exposed to similar doses of hydergine and except complete blood counts, other parameters determined were as in the *in vivo* experiments.

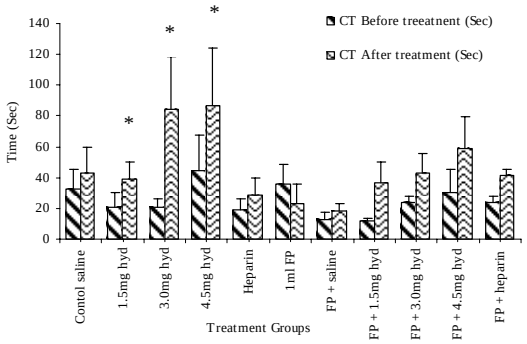
D. Statistical Analysis

One-way analysis of variance (ANOVA) was applied (Statistical Package for Social Sciences for windows SPSS version 10.0) to compare the variation among different parameters in hydergine treated animals in comparison to those of saline treated control. Post hoc Tukey's and Dunken's tests were applied for intra-group variation. Paired t-test was applied (Sigma Stat 2.0, Jandal Scientific USA) to verify the difference in the bleeding and clotting time of animals before and after drug introduction. Wilcoxan signed rank sum test was applied to those groups where normality test failed. A probability value of $P<0.05$ was considered significant difference.

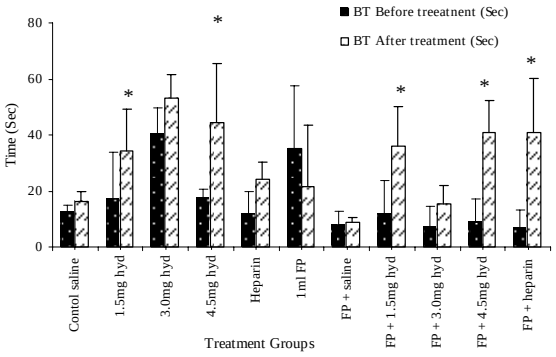
III. Results

In general, hydergine treated rats were sluggish and diarrhea was the outcome. Their tails cut for the estimation of CT and BT started bleeding again about half an hour after the drug introduction and the eyes of some of the acutely exposed animals were hemorrhagic at the site of lacrimal caruncle. Additionally, blood plasma of some (40%) of the animals who were administered 1.5 mg hydergine did not clot at all even though it was treated with APTT reagent.

The CT of hydergine treated animals increased significantly ($P<0.001$). It was prolonged in all the three treatment regimens as compared to the control group of rats and to the group exposed to fresh plasma before treatment with hydergine (Fig. 1A). Similarly, the BT of the rats exposed to 1.5 mg and 4.5 mg hydergine with and without prior fresh plasma was significantly prolonged ($p<0.001$) after the treatment and was comparable to the positive control animals treated with heparin+fresh plasma. The positive controls administered with fresh plasma alone showed a significant drop of bleeding time (Fig.1B).



(a)



(b)

Fig. 1 CT (a) and BT (b) of rats before and after treatment with different doses of hydergine, heparin and fresh plasma. [hyd: Hydergine, FP: Fresh plasma. *Significantly different from saline control ($P<0.05$)]

Plasma calcium concentration was significantly decreased ($P<0.001$) only in those rats who were exposed to 4.5 mg hydergine alone. Treatment with hydergine at all dose regimens along with prior exposure to fresh plasma also caused a decline in the plasma calcium concentration. A similar effect was obtained in the positive control animals treated with heparin either with or without prior exposure to fresh plasma.

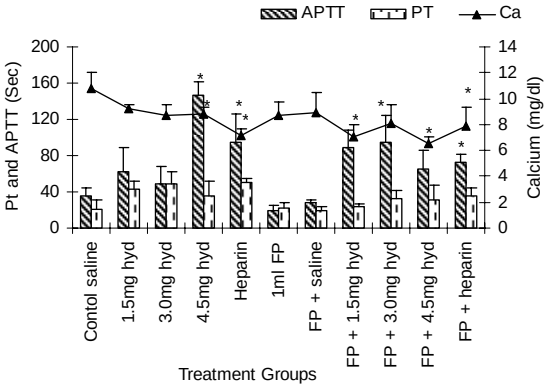
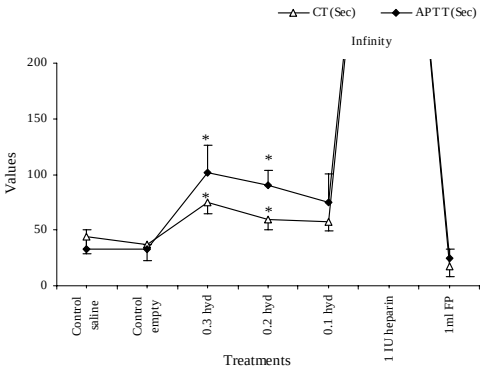
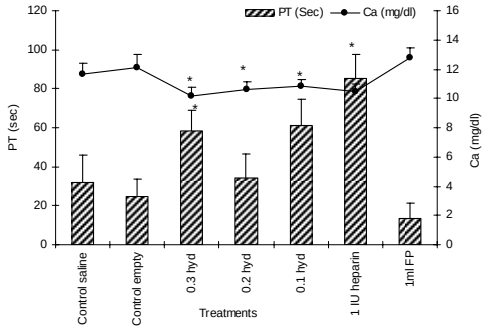


Fig. 2 PT, APTT and plasma calcium of rats treated with different doses of hydergine, heparin and fresh plasma. [hyd: Hydergine, FP: Fresh plasma. *Significantly different from saline control (P<0.05)]

Ex vivo, CT and APTT of the blood samples exposed to 0.3 mg and 0.2 mg hydergine were significantly prolonged (P<0.001) whereas samples to which heparin was added did not clot at all (Fig. 3A).



(a)



(b)

Fig. 3 CT and APTT (a) PT and plasma calcium levels (b) of the plasma exposed to different doses of hydergine, fresh plasma and heparin in *ex vivo* experimentation system. [hyd: Hydergine, FP: Fresh plasma. *Significantly different from saline control (P<0.05)]

PT of the blood samples exposed to 0.3 mg hydergine and heparin *ex vivo* were significantly (P<0.05) elevated than the control blood given no exposure of any kind. The plasma calcium concentration of the blood samples exposed to different concentrations of hydergine was significantly reduced (P<0.001) as compared to those treated with fresh plasma where an elevation of calcium levels was observed (Fig. 3B).

IV. DISCUSSION

Adverse events such as recurrence of bleeding associated with antithrombin therapies although have largely negated their potential benefits; antiplatelet therapy has modestly improved the outcome in both acute stroke and secondary stroke prevention [8]. Despite the fact that progress has been made in developing low molecular weight heparin, powerful and effective anticoagulants still remain to be identified. Equally, there is also a need for safer oral anticoagulants that do not require routine coagulation monitoring [9].

Earlier studies have shown that in the hospitalized patients with acute coronary artery disease, the ideal anticoagulant should be delivered by intravenous or subcutaneous injections and dosed easily so that it does not require frequent monitoring and can be reversed immediately and predictably. Rapid reversal of drug activity can be achieved by formulation of a drug as an infusible agent with a short half-life or by administration of a second agent, an antidote that can neutralize the activity of a drug [10]. It should be noted that the removal of hydergine from blood begins 1.5 hrs after its administration and lasts for about 15 hours [5]. Hydergine, according to our study, presents another anticoagulant of choice but before this, stringent case control studies on human subjects are required to be done.

Presently an increase in the CT and BT as a result of treatment with high doses of hydergine was dose dependent and appears parallel to heparin which is well-known to significantly prolong the BT in a dose dependent manner. Thus, hydergine treatment led to a possible inhibition of the coagulation system. In this context, the dissociation of antithrombin and antihemostatic effect indicates that inhibition of the coagulation system at its initial stage is a promising approach for the development of new anticoagulants [11]. PTT of the rats treated with 4.5 mg hydergine was significantly prolonged than other treatment groups where no significant difference was observed. Moreover, high doses of hydergine did not produce any significant effect on INR that was carried out to evaluate PT suggesting that the doses used presently were safe. It should be considered that in patients with thrombosis, PT is not significantly influenced by safe dose of heparin [12]. The effect of hydergine therefore seems equivalent to heparin. Since calcium levels declined with hydergine treatment both *in vivo* and *ex vivo*, it is suggested here that hydergine may prolong blood clotting possibly by lowering calcium concentration via modulating calcium release.

The decreased clotting efficiency where animals were exposed to fresh plasma before treatment with hydergine is an effect similar to a previous study where the addition of fresh frozen plasma to whole blood *in vitro* after systemic heparinization significantly prolonged the activated clotting time [13]. In this respect, hydergine appears similar in its activity to another cognition enhancer; *Ginko biloba* that has been shown to inhibit platelet function [14]. Ginger also

contributes to spontaneous bleeding if combined with anti-platelet anticoagulant drugs such as coumadin. Likewise, when combined with coumadin, ginseng can increase the risk of bleeding problems [15]. Studies like these however do not exist as per se regarding hydergine and therefore necessitate further evaluation.

Prolonged CT as a result of *ex vivo* exposure of blood to hydergine implicates a strong thrombolytic activity of the drug. It has been demonstrated that the determination of CT by free oscillation rheometry of blood samples collected in vacutainer or vacuette tubes that were recalcified, decreased after 30 minutes with maximal effect [16]. Our study concludes that hydergine is functionally equivalent to an ideal anticoagulant and suggests that it may be useful for the treatment of the disorders related to excessive clotting (thrombosis).

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