## 1209 AFFINITY LABELING OF HUMAN THROMBINS WITH EXTENDED BENZAMIDINE SULFONYLFLUORIDE EXO-SITE REAGENTS

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The reagent m-[o-(2-chloro-5-fluorosulfonylphenylureido)phenoxybutoxy]benzamidine [mCP(PBA)-F] is an exo-site affinity labeling reagent for human thrombins. To delineate the sites of the reaction of mCP(PBA)-F on thrombin (Th), experiments were done with three structurally related variants of mCP(PBA)-F in which the distance from the cationic amidino portion to the reactive sulfonylfluoride was varied by 5-6 A. In clotting inhibition assays the reagent m-[o-(2-chloro-5-fluorosulfonylphenylamido)phenoxypropoxy]benzamidine ($t_{½}$ = 99.4 ± 7.8 sec, $k_{1st}$ = 6970 ± 550 x $10^6$ $sec^{-1}$ n=5) was 2-fold more inhibitory than mCP(PBA)-F ($t_{½}$ = 200 ± 10.1 sec, $k_{1st}$ = 3460 ± 150 x $10^{-6}$ $sec^{-1}$ n=5). Minimal energy models of the compounds show the optimum length is 14-17 Å. In other experiments hirudin (9600 µ/mg, gift F. Markwardt) mixed with 13.1 µM α (clotting) or 12.1 µM γ/β (nonclotting) Th in 1- to 3-fold molar excess did not prevent incorporation of [$^3$H]mCP-(PBA)-F (0.961-0.794 mole label/mole Th) but did inhibit [$^3$H]diisopropylfluorophosphate ($iPr_2P$-F) uptake (.096-.005 mole label/mole Th). mCP(PBA)-Th had reduced binding for proflavin (Kd ≃ 9-fold that of Th) but not as low as $iPr_2P$-Th (Kd ≃ 38-fold that of Th) and further suggests that mCP(PBA)-F is attached to Th at points distal to the catalytic site. These new data show that mCP(PBA)-F reacts at nonclotting related but specific secondary sites on Th and that mCP(PBA)-F can be used to identify the structural features associated with the unique proteolytic specificity of thrombin. (Supported by NIH and AHA)

## 1210 DRUG PROPHYLAXIS OF THROMBOEMBOLIC COMPLICATIONS, LIMITS AND FACILITIES IN HIP SURGERY

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In 1973 we started prospective, controlled trials on prophylactic efficiency of 5 drugs: combination of aspirin and dipyridamol (95 patients), dextran 60 (43 patients), dihydroergotamin (61patients), low-dose-heparin (63 patients) and a combination of dihydroergotamin and low-dose-heparin two times (61patients), three times (63 patients) a day. The 386 patients undergoing total hip replacement were screened with the 125-J-fibrinogen-uptake-test, phlebography and a careful clinical evaluation before and after surgery. A lung perfusion scan was performed in the last 4 groups. In cases of established DVT simultaneous anticoagulation with heparin and coumarin was started. In the group with aspirin/dipyridamol prophylaxis 32 (34 %) DVT and 3 pulmonary emboli were detected. In the dextran group 24 (56 %) got DVT and one non fatal PE. The dihydroergotamin group showed 33 DVT (54 %) and 7 PE. In the low dose heparin group we had 29 (46 %) DVT and 3 PE. Only the combination of low dose heparin and dihydroergotamin reduced the incidence of thromboembolic complications significantly: no PE, 15 DVT (25 %). The last group showed no further reduction but more hemorrhagic complications.

## 1211 CHANGES IN THE PLASMA LEVEL OF HEPARIN AND COAGULATION FACTORS DURING THE POSTOPERATIVE PROPHYLAXIS WITH HEPARIN AND HEPARIN-DIHYDROERGOTAMIN.

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In a randomised prospective study a homogenous total of 70 women undergoing abdominal hysterectomy and bilateral adnexectomy is subdivided in 7 different groups with the following treatment schadules: A. no postoperative prophylaxis of thromboembolism, B. every 12 hrs 0.5 mg dihydroergotamin (DHE), C. every 12 hrs 2500 I.U. Na-heparin + DHE, D. every 12 hrs 5000 I.U. heparin, E. every 12 hrs 5000 I.U. heparin + DHE, F. every 8 hrs 5000 I.U. heparin, G. every 8 hours 5000 I.U. heparin + DHE.

The following parameters are examined pre- and postoperatively as well as on the first, second and seventh postoperative day prior to and 3 hrs after the respective treatments: plasma-levels of heparin, anti Xa and antithrombin III with chromogenous substrate 2160 and 2222, PTT and plasma thrombin time as well as the factors of the coagulation and fibrinolytic system.

The preliminary data indicate that under the combined administration of heparin plus dihydroergotamin heparin levels rise further and PTT is more prolonged as compared to heparin prophylaxis alone.