filtered, made alk with cold aq NaOH, and extd thoroughly with Et_2O. The crude product was recrystd from hexane, giving 7.9 g (83%) of I, mp 72.5–73.5° (hexane), lit.[2] 73–74°, nmr (CCl_4) 6.62, 6.57 (H 3, H 5), 3.76 (s, CO_2CH_3), 2.04 (s, CH_3).

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Fibrin-Stabilizing Factor Inhibitors. 4. Action of Some Synthetic Fibrinoligase Inhibitors on Human Platelet Aggregation[1]

Ragnar Lundén, Ann Hartkoorn,

AB Kabi, Fack, S-104 25 Stockholm, Sweden

J. Lars G. Nilsson,* Pål Stenberg,

Faculty of Pharmacy, University of Uppsala, S-113 86 Stockholm, Sweden

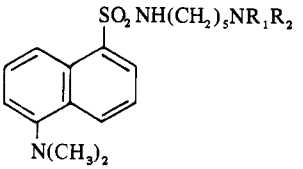
Joyce Bruner-Lorand, and Laszlo Lorand

Northwestern University, Biochemistry Division, Department of Chemistry, Evanston, Illinois 60201. *Received June 28, 1971*

It has been shown that fibrinogen plays a role in the aggregation of platelets.[2] Therefore, we thought it would be of interest to examine the possible effect on platelet aggregation of compds which are known to inhibit specifically the enzymatic cross-linking of fibrin. In plasma, the cross-linking of fibrin is the last step in normal blood coagulation, and the reaction is catalyzed by fibrinoligase, a transamidase, which arises from the fibrin-stabilizing factor zymogen (factor XIII) through activation by thrombin.[3] The presence of a similar,[4] though not identical,[5] transamidase in platelets is well known and the requirement for thrombin has also been shown.[6]

Monodansylcadaverine [*N*-(5-aminopentyl)-5-dimethylamino-1-naphthalenesulfonamide (1)] is one of the most effective inhibitors of fibrinoligase.[1,7] We found that this compd also inhibited the second phase of ADP-induced aggregation of human platelets (Figure 1), whereas the initial phase was only slightly or not at all affected.

It was then pertinent to ascertain whether the effect of monodansylcadaverine on the platelet aggregation was due to its inhibitory effect on fibrin cross-linking, *i.e.*, whether it was dependent on its functional primary amino group. While several primary amines (*e.g.*, glycine methyl ester) inhibit the fibrinoligase-catalyzed reaction, the corresponding *N*-alkyl derivs (*e.g.*, sarcosine methyl ester) are ineffective.[7a] We have also shown[1] that the similarly alkylated dansyl compds 2 and 3 have no inhibitory action on fibrinoligase.



1, $R_1 = R_2 = H$
2, $R_1 = H$; $R_2 = CH_3$
3, $R_1 = R_2 = C_2H_5$

Aggregation experiments were performed on a number of plasma samples as described in the Experimental Section. Figure 1 gives a typical example of the recordings. All 3 compds showed an inhibitory effect on the second phase of the ADP-induced platelet aggregation. There is some suggestion that monodansylcadaverine (1) may be more effective than the two analogs (2, 3).

Inhibition by monodansylcadaverine was even more pronounced when norepinephrine was used to initiate platelet aggregation (Figure 2). In this case, already the first phase of aggregation seemed to be inhibited.

It can thus be concluded that monodansylcadaverine inhibits both the ADP- and norepinephrine-induced aggregation of human platelets. Since the compds with alkylated aliphatic amino groups (2, 3) act similarly, the primary amino group which is essential for inhibiting fibrin cross-linking, does not seem to be involved in the inhibition of platelet aggregation. Similar alkylated amines have recently been studied by Lacefield, *et al.*,[8] and shown to inhibit platelet aggregation.

Experimental Section

Biological Methods and Materials. The method of Born was followed for measuring platelet aggregation,[9] using an EEL titrator equipped with a magnetic stirrer and thermostated at 37° (±0.2°). The titrator was coupled to a Labograph E 478, Methrohm AG recorder.

All glassware used in contact with blood or plasma samples was siliconized. Fresh human blood was drawn into 3.8% sodium citrate

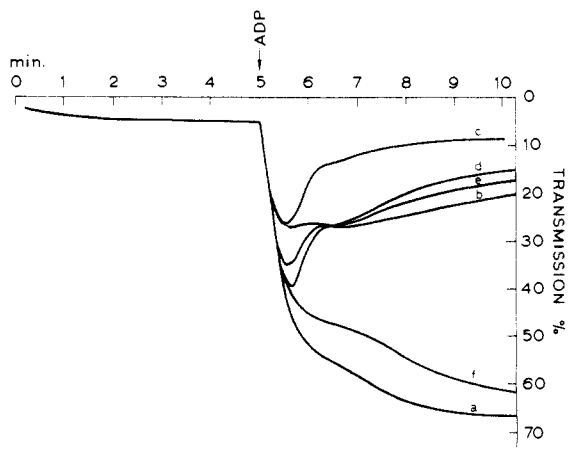


Figure 1. Effects of dansylcadaverine (1) and 2 and 3 on ADP-induced platelet aggregation. Inhibitor, $3.1 \times 10^{-4} M$; ADP, $3.1 \times 10^{-6} M$. Curve a and f: no inhibitor, a recorded at the start and f at the end of the expt. Curve: b, adenosine ($3.1 \times 10^{-6} M$); c, dansylcadaverine (1); d, compd 2; e, compd 3.

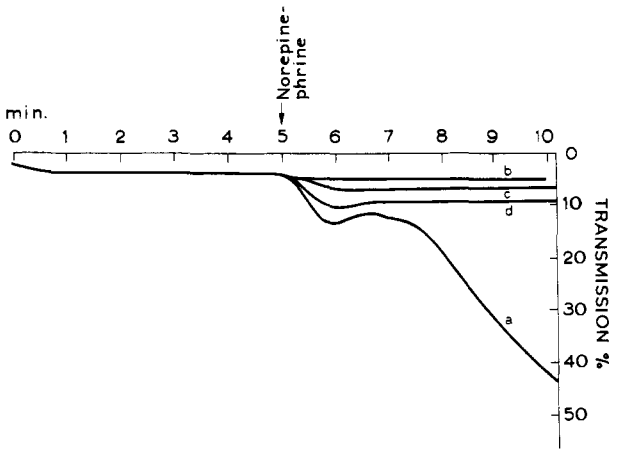


Figure 2. Effect of dansylcadaverine (1) on norepinephrine-induced platelet aggregation: a, no inhibitor; b, $3.1 \times 10^{-4} M$; c, $1.6 \times 10^{-4} M$; d, $6.2 \times 10^{-5} M$. Norepinephrine, $6.2 \times 10^{-6} M$.

soln (1–9 ml of blood). The blood was centrifuged at 200*g* for 15 min to prep platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtd by centrifugation for 20 min at 3200*g*. All these operations were performed at room temp and completed within 45 min after the blood had been drawn. Platelets were counted using a Büchner chamber and a phase contrast microscope. Counts for PRP varied between 300,000 and 500,000 cells/mm³.

Adenosine diphosphate (ADP) Na salt (Sigma Chemical Co.) was dissolved in Tris buffer (0.05 *M*) to a concn of 5×10^{-3} *M*. The soln was divided into aliquots and stored at −70°. Norepinephrine (Sigma Chemical Co.) was made up to $6.2 \times 10^{-6} M$ immediately before the expt and was kept at 0° until used.

The aggregation expts were carried out as follows. PRP (3 ml) and PPP (3 ml) were transferred into 2 sep cuvettes. The recorder was adjusted to 95% transmission against PPP and to 5% transmission against PRP; 0.1 ml of saline (0.08 *M* NaCl) was added to the cuvette contg PRP. It was allowed to stand in the titrator with stirring for 5 min, when aggregation was initiated by adding 0.1 ml of the ADP soln.

In the expts with inhibitors, saline was replaced by 0.1 ml of a soln of one of the compds 1–3, dissolved in the Tris buffer. Adenosine (Sigma Chemical Co.) was employed as a "standard" inhibitor.

A "blank recording" using saline instead of inhibitor soln was run in the beginning and the end of each exptl series so that the effects of storing PRP could be observed. All expts were completed within 3–4 hr after drawing the blood.

For each plasma sample the suitable ADP concn had to be detd. A sufficiently low concn was chosen to give a typical two-phase curve showing a second wave of platelet aggregation. This concn was usually of the order of 10^{-6} *M*.

Chemical Methods and Materials. Melting points were detd with calibrated Anschütz thermometers in an electrically heated metal block. Ir spectra were run for identification purposes on a Perkin-Elmer 237 spectrophotometer. New compds, which were analyzed for C, H, and N, gave values within ±0.4% of the theoretical ones. Dansyl chloride was commercially available.

5-Benzylmethylaminopentylamine. 5-Benzylmethylaminovaleronitrile[10] (5 g; 24.7 mmoles) was reduced in 100 ml of dry Et_2O using LAH (1.14 g; 30 mmoles). The mixt was refluxed for 6 hr and worked up as usual yielding 4 g of product, bp 114–115° (1.5 mm) [lit.[10] 107–109° (0.5 mm)].

***N*-(5-Benzylmethylaminopentyl)-5-dimethylamino-1-naphthalenesulfonamide.** This compd was prepd in 85% yield from dansyl chloride and 5-benzylmethylaminopentylamine as previously described for **1**. The dihydrochloride had mp 175° dec (from EtOH–Et_2O). *Anal.* ($C_{25}H_{33}N_3O_2S \cdot 2HCl$): C, H, and N.

***N*-(5-Methylaminopentyl)-5-dimethylamino-1-naphthalenesulfonamide (2).** The preceding benzylamino deriv (1 g; 2.3 mmoles) in EtOH (50 ml) was hydrogenated over 0.1 g of 10% Pd/C at room temp overnight in a Parr app with an initial H_2 pressure of 3 kg/cm². After filtration and evapn, the amine (0.5 g; 63% yield) was converted to its dihydrochloride, mp 245–248° (from EtOH-ether). *Anal.* ($C_{18}H_{27}N_3O_2S \cdot 2HCl$): C, H, and N.

***N*-(5-Diethylaminopentyl)-5-dimethylamino-1-naphthalenesulfonamide (3).** This compound was prepared in 59% yield from dansyl chloride and 5-diethylaminopentylamine as previously described for **1**.[1] The dihydrochloride had mp 168–170° dec (from EtOH–ether). *Anal.* ($C_{21}H_{33}N_3O_2S \cdot 3HCl$): C, H, and N.

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Substituted Thiazolidones and Their Selective Inhibition of Nicotinamide-Adenine Dinucleotide Dependent Oxidations†

Surendra S. Parmar,* C. Dwivedi,‡ A. Chaudhari,§ and T. K. Gupta

Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow-3, India.
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CNS depressants that have profound effects on the activity of the brain are believed to inhibit certain metabolic processes.[1,2] Interactions with receptor surfaces[3] may account for structure-activity relationships of various psychopharmacological agents. In the present study the ability of thiazolidone derivatives to exhibit a wide variety of pharmacological properties, including anticonvulsant activity,[4,5] led us to investigate the ability of such thiazolidones to inhibit oxidation of the substrates of the tricarboxylic acid cycle like pyruvate, α-ketoglutarate and citrate, and β-hydroxybutyrate with a view of studying their biochemical mechanism of action. The anticonvulsant activity of these compounds was determined to correlate pharmacological properties with their enzyme inhibitory properties. The various substituted thiazolidones have been synthesized according to the methods outlined in Scheme I.

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‡Junior Research Fellow of I.C.M.R., New Delhi.
§Junior Research Fellow of State C.S.I.R., Lucknow.