Spontaneous Hemorrhage Precipitated by an Emotional Stress Drug in Anticoagulant-Treated Rats*

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Emotional stress plays an important role with other factors in the spontaneous bleeding of patients with hemorrhagic disorders. Pavlovsky (20) on the basis of extensive work in hemophilics has interpreted the spontaneous hemorrhagic episodes as an alteration of the vascular tree produced by emotional factors, because the appearance and disappearance of these hemorrhagic manifestations are independent of the coagulation defect and related to psychic influences. Thus, Castex (3) described two hemophilics who suffered spontaneous hemorrhage following an emotional upset as a consequence of an automobile accident suffered by their father. The same two brothers often underwent severe hemorrhagic crises for several days before celebration of their birthdays. Other authors have pointed out the importance of emotional stress as a cause of spontaneous bleeding in hemophilics. Browne et al. (2) indicated that emotional factors can contribute to the timing of spontaneous bleeding in hemophilics. Poinsard (21) has stated that an emotionally tranquil hemophilic in a state of well-being bleeds less frequently and less severely, than the subject who is emotionally distressed, while Spaet's (24) states "it is common clinical experience that there is poor correlation between the clinical severity of the disorder (hemophilia) and the degree of prolongation of the clotting time; and that although the obvious medical disability is great, social and emotional disabilities often dominate the picture". The importance of controlling emotional factors in patients with blood dyscrasias undergoing dental extraction has also been pointed out (11, 12). Removal of fear and anxiety by hypnosis in hemophilics scheduled for a dental extraction has been shown to be excellent ad-

*) Assisted by the Cardeza Foundation, Jefferson Medical College and Hospital, Philadelphia, Pennsylvania and by the United States Public Health Service Research Grant HE-04877. We are indebted to Dr. A. J. Erslev and Dr. L. B. Kazal of Cardeza Foundation for their interest and support.

junctive therapy in effecting hemostasis after the extraction of a tooth even in a severe hemophilic (13).

In this study the relationship of emotional stress to hemostatic mechanisms in laboratory animals was examined by studying the influence of drug stressors as hemorrhagic agents in anticoagulant-treated rats. Previous work in this laboratory has clearly demonstrated that when a stressor such as 10% NaCl i.p., restraint, insulin shock, etc. is administered with an anticoagulant to rats or rabbits, a high incidence of spontaneous hemorrhage is observed (8, 25). Jaques defined this type of hemorrhagic manifestation as "Hemorrhage due to multiple causation" (7). D-Lysergic acid diethylamide (L.S.D.) was used in this study as an emotional stressor agent. Transitory disturbances, hallucinations, depersonalization, reliving of repressed memories and mild neurovegetative symptoms follow its administration in humans. L.S.D. was reported as a powerful stimulant of all parts of the central nervous system, mainly the cortical and hypothalamic centres. All parts of the central nervous system are affected to a greater or lesser degree, and the resulting changes in organs and functions may be marked. Gross overdosage also exerts its most characteristic toxic actions on the cortex and medulla (5). The action of L.S.D. starts about thirty minutes after administration and reaches a peak of general sympathetic excitation between the third and fourth hours and may last up to 12 hours (4, 16). Other authors have found in L.S.D. a strong specific antiserotonin activity (23, 26). Since serotonin is believed to play an important normal role in hemostasis (6, 19), the antiserotonin activity of L.S.D. may affect the hemostatic mechanism. A lowered capillary resistance in a small area of the skin which has been infiltrated by iontophoresis with L.S.D. has been reported (1). However, the main reason for using L.S.D. in this study is because of its action on the central nervous system as an emotional stress agent for rats. Since lysergic acid monohydrate (L.A.) has the same action on the pituitary-axis without its effects on the higher nervous system, L.A. was included in this study as a control.

Materials

Rats

Two hundred and forty Wistar strain, white, male rats of body weight ranging between 200–250 gm were used in this experiment. The rats were picked at random and kept in separate cages for the 10 day duration of the experiment.

Anticoagulant

Phenylindanedione (P.I.D.) was fed ad lib. during the experiment in two different concentrations, 1.5 mg per 0.75 gm, and 1 mg per 0.75 gm of powdered food on alternate days. We are indebted to Chas. E. Frosst & Company, Montreal, for the phenylindanedione.

D-Lysergic Acid Diethylamide (L.S.D.) was obtained from Sandoz Pharmaceuticals, Montreal, and given intraperitoneally, 0.2 mg in 2 ml per 200 gm body weight.

D-Lysergic Acid Monohydrate (L.A.) was obtained from Light & Co. Limited, London, England and given intraperitoneally 0.3 mg in 2 ml per 200 gm body weight.

Physiological saline was given intraperitoneally, 2 ml per 200 gm body weight as for L.S.D. and L.A.

Methods

L.S.D. was given alone to groups of 10 rats with a group receiving one i.p. injection on day 0, and another group receiving two injections, one on day 0 and the other one on the third day of the experiment. Two other groups received L.S.D. as above plus anticoagulant which was fed beginning on day 0. Following the administration of L.S.D., the first sign of response was noticed in approximately 20 minutes. It consisted of an increase of activity with the rat exploring the cage in a very excited manner. This excitatory period was sometimes interrupted suddenly by violent shaking of the entire body. Twenty to thirty minutes following the injection of L.S.D. the excitation waned and with the abdomen touching the floor of the cage the rat crawled around the cage. Following this state of reduced activity, the animals became extremely quiet and practically motionless in the corner of the cage. These findings agree very well with reports by others (27).

L.A. alone was given to a group of twenty rats. A group received one injection on day 0 and another group received two injections on day 0 and on the third day. Two other groups also received L.A. as above, plus anticoagulant which was fed beginning on day 0. Following the administration of L.A., no hyperactivity was observed as in the rats receiving L.S.D.

Physiological saline was also given to control groups of twenty rats in the same form as for L.S.D. and L.A. One group of twenty rats was fed anticoagulant alone during the experiment as a control in two different doses, 1.5 mg per 0.75 gm and 1 mg per 0.75 gm of powdered food on alternate days, beginning on day 0. The anticoagulant dose was about 25 mg per day. Another group of twenty rats without any treatment was used as control.

All animals dying during the experiment were examined as soon as possible after death. A post-mortem examination was made and all evidence of hemorrhage, or no hemorrhage, recorded. Tissues were removed for histological examination. However, all animals dying in the present series presented evidence of gross hemorrhage. At the end of the ten day period, animals were sacrificed with ether, and examined. Results are recorded in the tables as death from hemorrhage in the case of those animals dying during the experiment, and hemorrhage observed for those animals in which hemorrhage was found when they were sacrificed.

Results

As indicated in Table 1, a high incidence of hemorrhagic manifestations occurred when the stress agent was combined with anticoagulant therapy.

Two injections of L.S.D. with P.I.D. gave the highest percentage of mortality (70%) during the 10 day experiment. On post-mortem, the surviving three rats showed extensive hemorrhage internally as there was 100% incidence of hemorrhage in the group. One injection of L.S.D. with P.I.D. showed a 30% mortality during the experiment but hemorrhagic manifestations occurred in 80% of the group.

Table 1. Incidence of Hemorrhage in Rats Injected with L.S.D., L.A., Physiological Saline Receiving Anticoagulant.

Stress treatment	Anticoagulant (P.I.D.)	Total	No. of Rats		% with hemorrhage	
			Death from hemorrhage	Survivors hemorrhage	Dead	Total
L.S.D. 1×	+	10	0	0	0	0
L.S.D. 1×	+	10	3	5	30	80
L.S.D. 2×	+	10	0	0	0	0
L.S.D. 2×	+	10	7	3	70	100
L.A. 1×	+	20	0	0	0	0
L.A. 1×	+	20	4	12	20	80
L.A. 2×	+	20	0	0	0	0
L.A. 2×	+	20	2	14	10	80
Saline 1×	+	20	0	0	0	0
Saline 1×	+	20	1	5	5	25
Saline 2×	+	20	0	0	0	0
Saline 2×	+	20	3	7	15	50
—	+	20	0	0	0	0
—	—	20	0	0	0	0

One injection of L.A. with P.I.D. resulted in 20% of the rats dying during the experiment and 80% showing hemorrhagic manifestations. Two injections of L.A. with P.I.D., only caused 10% of the rats to die due to hemorrhage during the ten day experiment. The total incidence of hemorrhage in the group was 80%.

In the group where saline was given intraperitoneally, as for L.S.D. and L.A., as control groups, very low mortality occurred during the ten day experiment. In the group one injection of saline with P.I.D., mortality was only 5% and the total incidence of hemorrhagic manifestations in the group was 25%. In the group with two injections of saline plus P.I.D., 15% of the rats died due to hemorrhage and the total incidence of hemorrhagic manifestations was 50%.

In the control group receiving P.I.D. only and in the group with no treatment, not one of the rats died during the ten day duration of the experiment and no hemorrhagic manifestation was found in any rat when post-mortem was done in each individual rat on the tenth day.

Discussion

Previous studies in this laboratory (17, 25) have shown that various stress procedures do not affect the mean prothrombin times of normal or anticoagulant-treated rats, and the authors (14) have reported that this is also true for L.S.D. It can, therefore, be concluded that the spontaneous hemorrhages ob-

served are not due to the anticoagulant having a greater effect on the blood coagulation system.

As shown in the results, L.S.D., which is a strong emotional stressor in rats and acts as a powerful stimulant to all parts of the central nervous system, mainly the cortical and hypothalamic centres, and the pituitary-adrenal axis, caused extensive hemorrhage when combined with an anticoagulant. Death due to hemorrhage occurred in the groups with one and two injections of L.S.D. plus anticoagulant. However, mortality during the 10 day experiment was much

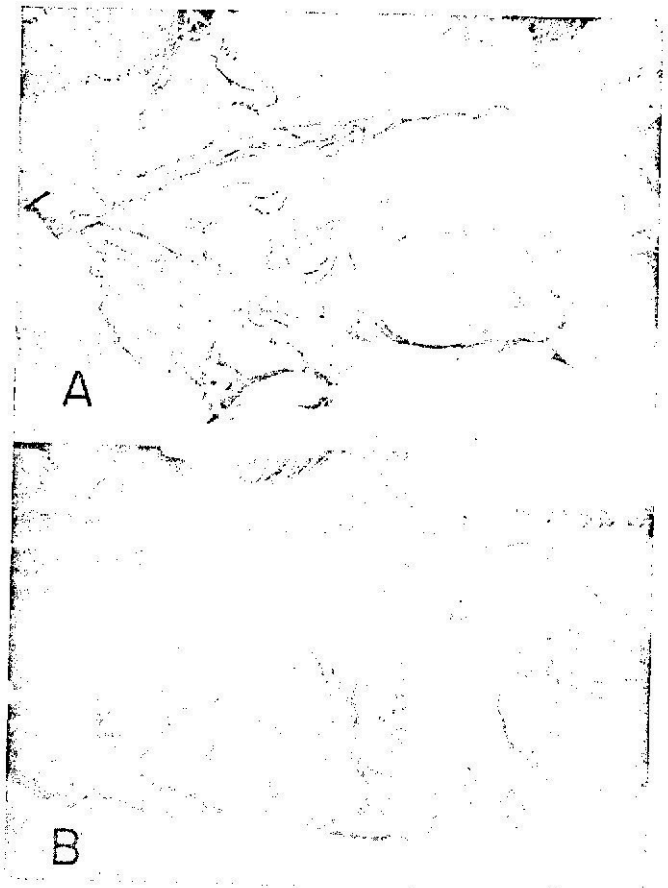

Fig. 1. Shows two rats that died during the 10 days of experiment from spontaneous hemorrhage, treated with one injection of L.S.D. on day 0 followed by anticoagulant ad lib.

Fig. 1A. Rat died on the 8th day, – note severe hemorrhage in pleural cavity, large amount of free blood.

Fig. 1B. Rat died on the 6th day; note severe hemorrhage in testicle, small, large intestine. Stomach presented a well defined hemorrhagic area.

Fig. 2. Two rats treated with two injections of L.S.D.; on day 0 and on the third day of experiment followed by anticoagulant ad lib.

Fig. 2A. Large amount of free blood in abdominal cavity; uro-genital tract and gastro-intestinal tract were severely involved. Rat died on the 8th day of experiment.

Fig. 2B. Uro-genital tract, severe hemorrhage – note severe hemorrhage in the dorsal wall of abdominal cavity involving the right kidney.

higher in the group with two injections. This may well indicate that death due to hemorrhage is related to the time during which the rat is exposed to the effect of L.S.D. The hemorrhagic manifestations in the rats that died during the experiment (Figs. 1 and 2), as well as the ones that were sacrificed on the tenth day, were in a variety of forms, with the uro-genital and gastrointestinal tracts being the systems more severely and most often involved. A few rats on post-mortem showed large amounts of free blood in the pleural (Fig. 1A) or abdominal cavity (Fig. 2A).

L.A. acts through the pituitary-adrenal axis without the effect on the central nervous system seen with L.S.D. The main reason for including L.A. in this experiment was to evaluate the influence of stimulation of the central nervous system as a cause of spontaneous hemorrhage. The results indicate that mortality was much higher in groups with L.S.D. than with L.A., and total incidence of hemorrhagic manifestations was higher with the two injections of L.S.D. with P.I.D. than in the case of L.A. with P.I.D. The fact that some deaths occurred in the groups where saline was given as for L.S.D. and L.A. plus anticoagulant may mean that the intraperitoneal injection itself is acting as a stressor agent in the rats. However, the clinical appearance during the experiment of the rats receiving saline plus P.I.D. was much better than that of the groups with L.S.D. plus P.I.D. No mortality or hemorrhage on post-

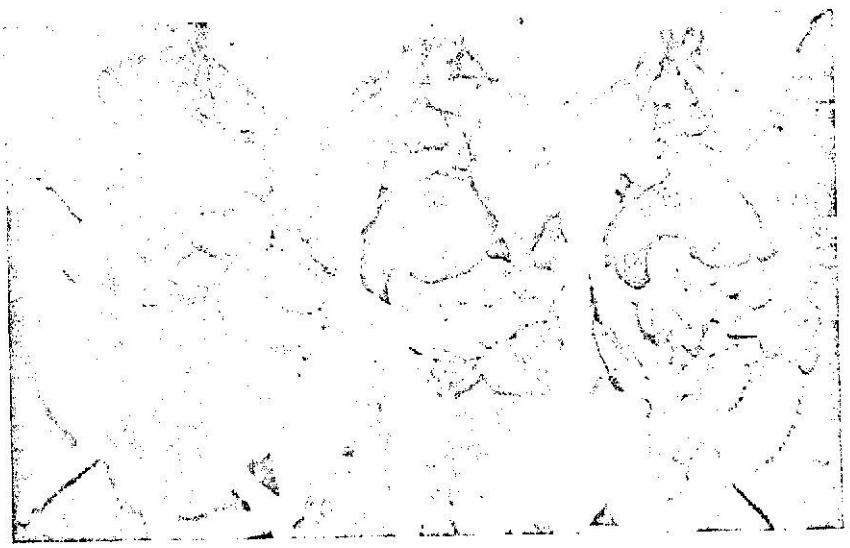

Fig. 3. Post-mortem appearance following sacrifice on the 10th day of experiment. Centre, normal rat; right and left rats received anticoagulant ad lib. during experiment.

mortem was recorded in the group receiving anticoagulant alone. The post-mortem appearance of these rats following sacrifice was compared with the group of rats that were not exposed to any kind of treatment (Fig. 3).

It is evident then that, when an emotional stress agent is given to rats in which one of the factors that participate in hemostasis is altered (in this case blood coagulation), high mortality due to spontaneous hemorrhage occurs and there is evidence of spontaneous hemorrhage in all the rats so treated. However, further work is required to determine if spontaneous hemorrhage under these conditions can be obtained by drugs which produce a high degree of central excitation without antiserotonin effect, as is the case for L.S.D.

Emotional stress as a cause of impairment in the different factors that participate in hemostasis has been reported. Castex considers that emotions may effect the vasomotor centres and be directly responsible for the alteration of the blood vessels that produce spontaneous hemorrhage in hemophilics (3). On the other hand, other authors have reported a direct relationship between emotional disturbances and an increase in the fibrinolytic system (15, 18, 22) and emotional stress and capillary response (9, 10).

Summary

L.S.D. was used as an emotional stress agent in rats in an attempt to evaluate the influence of the central nervous system in triggering spontaneous

hemorrhage in rats treated with anticoagulants. L.A. was used to evaluate whether the hemorrhagic activity of L.S.D. was through the pituitary-adrenal axis. A high percentage of spontaneous hemorrhage was found when the emotional stress agent was combined with the anticoagulant. There was no mortality or hemorrhagic manifestation with anticoagulant alone or in the groups where the treatments were given alone as a control.

Résumé

La diéthylamide de l'acide lysergique (L.S.D.) a été utilisée comme agent produisant un stress émotionnel chez le rat. Il s'agit d'une tentative d'évaluation de l'influence du système nerveux central dans le déclenchement d'une hémorragie spontanée chez le rat traité aux anticoagulants. L'acide lysergique a été employé pour savoir si l'activité hémorragique du L.S.D. se manifeste par l'intermédiaire du système hypophyse-surrénale. Une haute incidence des hémorragies est observée quand le stresseur est associé à l'anticoagulant. Il n'y a ni mortalité ni manifestation hémorragique si l'anticoagulation ou les autres traitements sont administrés isolément.

Zusammenfassung

L.S.D. wurde als ein psychischer Stressor bei Ratten verwendet, um die Auswirkungen des Zentralnervensystems auf die Auslösung spontaner Blutungen bei Antikoagulantienbehandlung der Ratten zu untersuchen. Lysergsäure-Monohydrat wurde verwendet, um zu erproben, ob die hämorrhagische Wirkung von Lysergsäure Diethylamid über das Hypophysen-Nebennieren-System ausgeübt wird. Ein hoher Prozentsatz von spontanen Blutungen wurde gefunden, wenn der emotionelle Stressor mit Antikoagulantien kombiniert wurde. Es waren keine Mortalität und keine Blutungen nachweisbar, wenn das Antikoagulans allein gegeben wurde oder bei jenen Tiergruppen, welche die Behandlung mit Lysergsäure Diethylamid allein als Kontrolle erhielten.

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