Brit. J. Pharmacol. (1961), 17, 392–395.

IMMEDIATE EFFECTS OF CATECHOLAMINES ON PLASMA FIBRINOGEN IN CONSCIOUS AND ANAESTHETIZED DOGS

BY

J. M. McKENZIE, L. J. O'BRIEN AND W. B. STAVINOHA

From the Civil Aeromedical Research Institute, Oklahoma City, Oklahoma

(*Received July* 24, 1961)

Adrenaline, noradrenaline, and isoprenaline were injected into dogs and the levels of plasma fibrinogen were estimated for up to 90 min. No changes in the concentrations of circulating plasma fibrinogen were found following the administration of any of the agents. These results do not support the view that hyperfibrinogenaemia occurs immediately following injection of adrenaline or that changes in fibrinogen concentration are related to alterations in blood pressure.

It has been reported that adrenaline produces a prompt and marked increase in the concentration of plasma fibrinogen in the dog (Rieker & Winters, 1931 ; Goreczky & Kováts, 1942). This hyperfibrinogenaemia occurred within 15 min following intravenous or subcutaneous injection of the drug. These results indicated the existence of a fibrinogen pool which was available for rapid mobilization by the autonomic nervous system. An inquiry into the mechanism of fibrinogen release from storage sites and the action of the common catecholamines seemed important in the light of other experiments which indicate that such compounds are not immediately effective in the rat (Henriques, Henriques & Levy, 1956). Thus, we have studied the response of dogs to the pure L-isomers of adrenaline and noradrenaline and to isoprenaline.

METHODS

Thirteen healthy mongrel dogs of both sexes, obtained from the pound, were used directly or kept for 27 to 55 days in individual cages in veterinary quarters. These latter animals were fed once daily a diet consisting of a commercial dog food mixed with cooked lean beef, and water was available at all times. The dogs were allowed to exercise daily.

The drugs employed in this study were (−)-adrenaline bitartrate (100 μg/kg in 1 to 2.5 ml. of solution), obtained from the Aldrich Chemical Co., Milwaukee 10, Wisconsin (m.p. 144° C), or the Winthrop Laboratories, New York, 18, New York (m.p. 149–150° C), and (−)-noradrenaline bitartrate (Levophed) (8 μg/kg in 0.08% solution) and isoprenaline hydrochloride (Isuprel) (5 μg/kg in a 0.05% solution), obtained from the Winthrop Laboratories, New York 18, New York. The diluent used was 0.85% sodium chloride solution. The drugs were tested in dogs which had received no anaesthetic and in others which were anaesthetized with pentobarbitone sodium or with a combination of thiopentone sodium and chloralose. In the latter procedure, thiopentone sodium (15 mg/ml.) was injected by vein until the animal became quiet enough to be given 100 mg/kg of chloralose by vein, which was dissolved in a minimum volume (usually 150 ml.) of warmed 0.85% sodium chloride solution. Each anaesthetized animal was connected through a tracheal cannula to a positive pressure respiration pump (Harvard Apparatus Company) and pulmonary ventilation was adjusted while monitoring arterial blood *p*H. Blood pressure was recorded from the left common carotid

artery by means of a Sanborn pressure transducer coupled to a Sanborn photographic oscillograph.

The right femoral triangle was dissected and the femoral artery was exposed distally for about 15 cm. Blood samples were withdrawn from this artery through 20-gauge needles by taking the first sample from the most distal segment and subsequent samples from more proximal segments. The drugs were injected into the right femoral vein. Blood pressure and heart rate responses indicated that the doses used were effective. In one animal, L-adrenaline bitartrate (97 μg/kg in 9.7 ml. solution) was infused over a 5 min period. Blood samples were withdrawn from unanaesthetized dogs via the left and right antecubital veins, and drugs were injected by the same route as well as by the subcutaneous route. All samples were immediately decalcified by mixing 9 parts of blood with one part of cold 4.0% trisodium citrate solution. This mixture was kept for 90 min or less in an ice-water bath and then centrifuged. Fibrinogen was estimated according to a semi-micro technique. Plasma (0.2 ml.) was clotted with thrombin (10 u.) in the presence of ice-cold phosphate buffer, *p*H 6.4. The resulting fibrin was wound out after 30 min incubation in an ice-water bath and dissolved in 0.1 N sodium hydroxide. Colour was developed with Folin-Ciocalteu reagent using L-tyrosine as a standard. An appropriate tyrosine-fibrinogen conversion factor (Johnston & Gibson, 1938) enabled a comparison to be made of results obtained by the Folin-Ciocalteu method with those obtained by a Kjeldahl technique. Triplicate analyses were performed on each sample.

RESULTS

Upon injection of 100 μg/kg of adrenaline, blood pressure in 5 animals changed from a mean of 145 mm mercury to a mean of 276 mm mercury and returned to the control value within 10 min. Average heart rate changed from 162 beats/min to 255 beats/min and returned to control values within 30 min. The effect of the injection of 8 μg/kg of noradrenaline was established in experiment 1 ; the mean blood pressure changed from 123 mm mercury to 178 mm mercury and returned to normal within a 30 min period. There was no change in heart rate. The effect of the injection of 5 μg/kg of isoprenaline was established in experiment 3 ; the mean blood pressure fell from 107 mm mercury to 48 mm mercury and returned to normal within a 10 min period. Heart rate increased from 156 to 312 beats/min and returned to near control value within 20 min.

Data for fibrinogen levels for each experiment are presented to emphasize the constancy of the results obtained. (Experiments are not numbered consecutively.) Fig. 1 presents the fibrinogen values obtained from animals which were injected with L-adrenaline. In experiments 4 and 5 the anaesthetic used was pentobarbitone

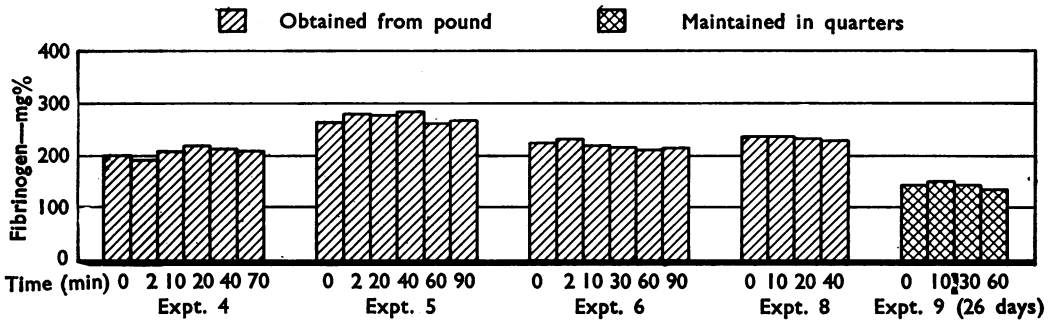

Fig. 1. Plasma fibrinogen levels in anaesthetized dogs after administration of adrenaline bitartrate, 100 μg/kg, in 1 to 2.5 ml. of solution.

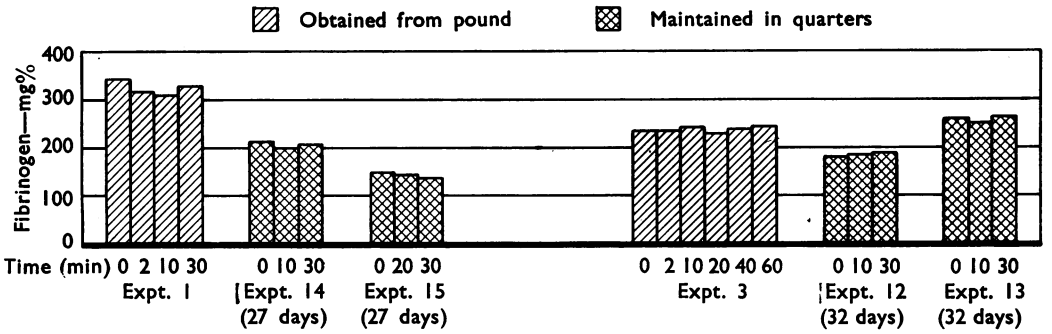

Fig. 2. Plasma fibrinogen levels in anaesthetized and conscious dogs after administration of noradrenaline, 8 μg/kg (expts. 1, 14 and 15), and isoprenaline, 5 μg/kg (expts. 3, 12 and 13).

sodium; in experiments 6, 8 and 9 the anaesthetic agents were thiopentone sodium and chloralose. Fig. 2 shows the results obtained when noradrenaline and isoprenaline were injected. The anaesthetic used in experiments 1 and 3 was pentobarbitone sodium; no anaesthetic was used in experiments 12, 13, 14 and 15. Inspection of the control values revealed that with one exception fibrinogen was lower in dogs that had been housed in veterinary quarters (mean is 185 mg/100 ml.) than in those obtained directly from the pound (mean is 249 mg/100 ml.).

In two unanaesthetized dogs, a mixture of adrenaline and noradrenaline was injected subcutaneously (Fig. 3). Dosage given was 80 μg/kg of adrenaline and 20 μg/kg of noradrenaline.

Thus it was found that injections of adrenaline, noradrenaline, isoprenaline or a combination of adrenaline-noradrenaline produced no immediate effects on plasma fibrinogen, although the doses employed produced profound circulatory changes.

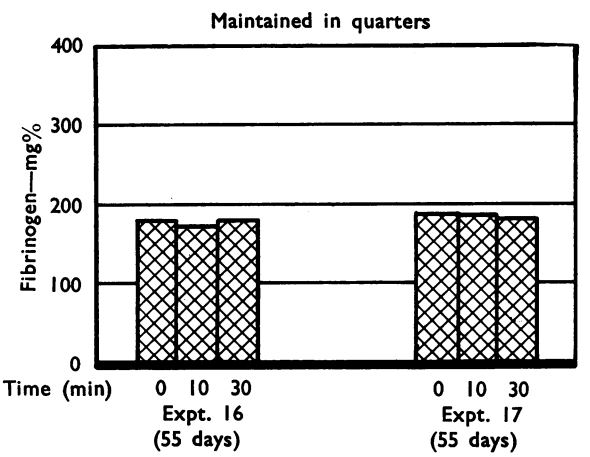

Fig. 3. Plasma fibrinogen levels in conscious dogs, maintained in quarters, after administration of a mixture of adrenaline, 80 μg/kg, and noradrenaline, 20 μg/kg.

DISCUSSION

It has been repeatedly demonstrated that marked changes in the level of plasma fibrinogen may occur in a variety of circumstances (Homburger, 1945 ; Gilchrist & Tullock, 1952). These changes are, however, slow in onset and may be due to increased synthesis or to shifts in body water. It has been suggested that the level of plasma fibrinogen is dependent, in part, on the level of blood pressure (Sütö-Nagy, 1944). The data obtained in these experiments do not support such an hypothesis in that no changes were obtained with the pressor or depressor agents used. An immediate rise in fibrinogen depends upon at least two prerequisites: fibrinogen must be stored in a labile pool and the drug used must be able to cause its release. It is well known that some anaesthetic agents are capable of depressing a number of physiological responses. However, our use of two different anaesthetic agents and the experiments with unanaesthetized dogs seem to rule out any likelihood that the drugs were prevented from acting by the conditions of the experiments. Furthermore, the chance that the fibrinogen pool had already been depleted by any stress factor as a result of dogs kept in a pound environment is obviated by the employment of animals housed for a time in veterinary quarters. The lower levels of fibrinogen in these latter dogs indicate that the level of circulating fibrinogen is dependent, in part, upon the state of nutrition and environment of the animals.

Because early preparations of adrenaline are known to have been mixtures of adrenaline and noradrenaline, a mixture was used in two experiments, but the results were identical with the previous experiments. Therefore, on the basis of the experiments reported in this paper, we conclude that exogenous adrenaline, noradrenaline, and isopropylnoradrenaline, and thus probably the sympathetic nervous system, are not involved in an immediate increase in plasma fibrinogen as previously reported (Rieker & Winters, 1931 ; Goreczky & Kováts, 1942). Our experiments do not, of course, eliminate the possibility that these catecholamines are involved in a slow fibrinogen response.

REFERENCES

GILCHRIST, E. & TULLOCK, J. A. (1952). Observations on the plasma fibrinogen content after myocardial infarction. *Edinburgh med. J.*, **59**, 561–567.

GORECZKY, L. & KOVÁTS, J. (1942). Über die Rolle der Lunge im Fibrinogenhaushalt. *Z. ges. exp. Med.*, **110**, 512–517.

HENRIQUES, S. B., HENRIQUES, O. B. & LEVY, A. (1956). Slow hyperfibrinogenaemic action of adrenaline and related substances. *Brit. J. Pharmacol.*, **11**, 99–103.

HOMBURGER, J. (1945). A plasma fibrinogen-increasing factor obtained from sterile abscesses in dogs. *J. clin. Invest.*, **24**, 43–45.

JOHNSTON, G. W. & GIBSON, R. B. (1938). The determination of blood plasma and spinal fluid proteins. *Amer. J. clin. Path.*, **8**, Supp. 2, 22–31.

RIEKER, H. H. & WINTERS, M. (1931). Influence of adrenalin on fibrinogen. *Proc. Soc. exp. Biol., N.Y.*, **28**, 671–674.

SÜTÖ-NAGY, G. J. (1944). Influence of acute changes in blood pressure on the distribution of fibrinogen. *Proc. Soc. exp. Biol., N.Y.*, **57**, 284–286.