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Pharmacological Induction of Fibrinolytic Activity in the Dog.* (28913)

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Activation of the fibrinolytic system with the aim of inducing thrombolysis can be produced in man by intravenous injection of exogenous material such as activator-free plasmin(1) or plasminogen activators, streptokinase and urokinase(2). The use of the body's own activators for thrombolysis has been poorly explored, though it has been repeatedly demonstrated that a variety of stimuli including pharmacological agents induce in man a marked rise of fibrinolytic activity. This is thought to be due to release of activator into the blood stream. Detailed exploration of this phenomenon has not been undertaken, primarily due to the lack of an appropriate experimental animal. In this paper it will be shown that pharmacological stimuli induce marked fibrinolytic activity in the dog.

Material and methods. Measurement of fibrinolytic activity. The lysis time of the euglobulin fraction prepared from citrated plasma was measured(3). Siliconized glassware was employed throughout the study. An intravenous catheter was inserted through a 14 G needle (Venocath™-14 Surgical 15 G, 8½'''', Abbott) into the jugular vein, and kept open by a slow saline infusion. The blood was withdrawn by the 2-syringe technique into a 5 ml syringe containing 1 ml of 3.8% sodium citrate USP. Tests were carried out within 45 minutes after shedding of the blood. Prolonged storage at any temperature tended to produce erratic results. The euglobulin lysis times were checked several times before and serially after i.v. injection of the compounds to be tested (Fig. 1A and 1B).

Animals and animal care. Healthy dogs of various breeds were obtained from the university-owned animal farm. In the laboratory the animals were kept for a few days in individual cages before the experiment. A diet consisting of either Frisky's canned dog food or of dog pellets mixed with horse meat and gravy was given.

Anesthesia. After taking an initial blood sample, all dogs were anesthetized with 26.5 mg/kg Diabutal® (Pentobarbital) intravenously. If required, additional smaller amounts of the anesthetic agent were given during the test. The anesthetized animal was lying on its back on a V-shaped dog board throughout the study.

Blood pressure recordings. After inserting a 16 G catheter into the femoral artery, the blood pressure and pulse rate were measured with Statham Strain Gauge (P23A) and visualized on a screen of a Hathaway Type MBC-2 oscillograph and recorded by a Heiland oscillogram on photo-sensitive paper.

Drugs. Carbachol (Delta Chemical Works, New York) 0.002% in saline, epinephrine (Parke, Davis, Detroit) 0.01%, and atropine sulfate (Mann Research Lab., New York) 10% in saline were used. The calculated doses were given intravenously in 60 seconds.

Results. Normal euglobulin lysis times. The average euglobulin lysis time before anesthesia in 22 healthy non-anesthetized dogs was 95 minutes, with variations from animal to animal as follows: 56, 64, 70, 72, 82, 82, 85, 88, 91, 95, 95, 95, 100, 100, 101, 101, 106, 108, 113, 116, 123, 142 minutes, S.D.

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19.89. The average euglobulin lysis time of newly arrived non-familiarized dogs kept in the dog pound of the animal farm was shorter: 62 minutes, and variations were greater as shown by the following: 31, 32, 32, 38, 39, 47, 47, 57, 63, 63, 66, 75, 79, 82, 87, 120 minutes, S.D. 24.41. Besides variations from animal to animal, there were also variations from day to day in the same dog as illustrated by the following figures. Dog #357: 10 samples, range 48-144, average 100 min; #830: 6 samples, range 115-156, average 144 min; #58: 5 samples, range 82-112, average 94.6 min; #551: 5 samples, range 55-108, average 81.6 min. The euglobulin lysis times were run daily for several days in dogs assigned to the study to eliminate those exhibiting particularly marked variations (standard deviations above 20 in most instances). Proper animal care tended to reduce but did not eliminate fluctuations of the euglobulin lysis time.

Anesthesia. To exclude any influence of emotional and physical stress on fibrinolytic system of the animals and to facilitate the drawing of serial samples, anesthesia was induced before onset of an experiment. The influence of Pentobarbital anesthesia on spontaneous fibrinolytic activity was minor. The average euglobulin lysis time of 20 dogs before induction of anesthesia was 96 minutes, S.D. 18.1, and during anesthesia 108 minutes, S.D. 23.9. Although there is a tendency to longer euglobulin lysis times after anesthesia, occasionally a minor reduction of the euglobulin lysis times was observed following intravenous Diabutal®.

Carbachol. a) Single injection: Intravenous injection of 0.01 mg/kg carbachol caused a very marked shortening of the euglobulin lysis time in non-anesthetized and anesthetized animals. Three dogs received this dose in the awakened state and on another day after induction of anesthesia. The resulting reductions of the euglobulin lysis time (expressed in percent) 5 minutes after intravenous injection are demonstrated by the following data: dog #148: –93% and –87%; #41: –87% and –89%; #237: –64% and –64%. The drop occurred within 2 minutes. It was preceded for about one minute by hyperperistalsis, defecation, urination, heavy breathing, excessive salivation, marked bradycardia and fall in blood pressure. The maximum shortening of the euglobulin lysis time lasted about 10 minutes and was followed by a gradual return to normal within one hour or less.

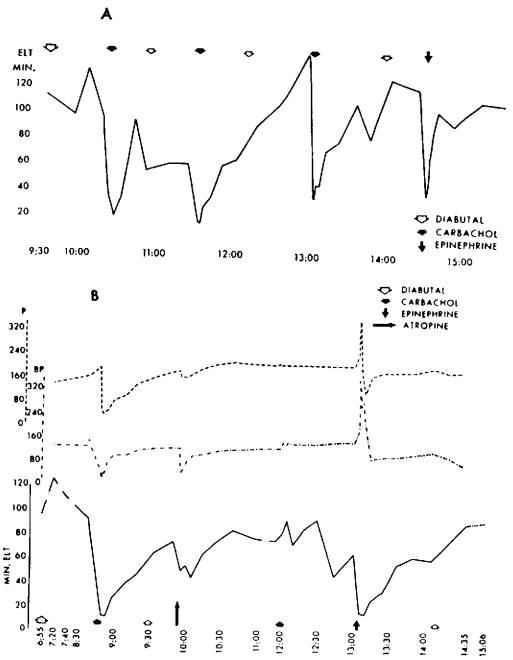

FIG. 1. A. Trend of euglobulin lysis time in the dog after repeated i.v. injections of 0.01 mg/kg carbachol followed by one i.v. injection of 0.025 mg/kg epinephrine. B. Trend of euglobulin lysis time, blood pressure, and pulse rate after successive injections of carbachol, atropine, carbachol, and epinephrine in the dog.

b) Repeated injections: Fig. 1A shows that the response to carbachol could be re-evoked several times per day. Carbachol was equally effective in reducing the euglobulin lysis time when given on subsequent days as shown by the data of dog #659; 10/15/62: –94%; 10/17: –87%; 10/19: –82%, and 10/20: –82%.

Atropine. As a first attempt to elucidate the pathway by which intravenous carbachol induces fibrinolytic activity, atropine, a parasympathomimetic blocking agent was given intravenously. Surprisingly, controls revealed that intravenous injection of 10 mg/kg atropine is immediately followed by a clear-cut reduction of euglobulin lysis time. The average reduction in 11 dogs 5 minutes after i.v.

atropine was −76% (range −55% to −89%, S.D. 11.25). Atropine injection, however, blocked both the pharmacological effect and the effect on the euglobulin lysis time of a subsequent carbachol injection (Fig. 1B).

Epinephrine. Intravenous injection of 0.025 mg/kg epinephrine reduced the euglobulin lysis time within 1 to 2 minutes to about the same extent as carbachol did. The patterns of the gradual return to normal were similar to those after carbachol (Fig. 1A and 1B).

Serial injections of different drugs in the same dog. A representative example of the effect of successive injections of carbachol, atropine, and epinephrine, given to the same dog is shown in Fig. 1B. Besides the marked action of all agents on the euglobulin lysis time it can be seen from this Figure that a preceding atropine injection essentially blocks the effect of carbachol on the fibrinolytic system. Fig. 1B reveals that rapid, marked change of the arterial blood pressure in either direction coincides with a marked drop of the euglobulin lysis time.

Discussion. Marked increase of fibrinolytic activity in man has been observed after subcutaneous injection of epinephrine(4), i.v. injections of acetylcholine(5), and i.v. injections of epinephrine, phenylephrine and norepinephrine(6). The mechanism of action is unknown. Animal studies with these or comparable compounds have not been reported with one exception(7): it was observed that 10 minutes after 0.13 to 0.49 mg/kg carbachol there was an increase of fibrinolytic activity of oxalated dog blood as measured with fibrin plate method. In this laboratory, these dog experiments could not be repeated because the animal died after i.v. injection of the reported dose. Furthermore, 0.07 mg/kg, the highest dose well tolerated in our hands, produced equivocal results on fibrin plates. With the euglobulin lysis time, however, the induced fibrinolytic activity was clear-cut. The carbachol studies further revealed: 1) The fibrinolytic enzyme system is very rapidly activated after intravenous injection, *i.e.*, within 2 to 3 minutes. This indicates that the injection is practically instantaneously followed by release of activator into the circulation. 2) The drug-triggered activation of the fibrinolytic system can be repeated either on several occasions on the same day or at daily intervals. There were no signs of tachyphylaxis. 3) The carbachol action on the fibrinolytic system is blocked by atropine which itself produces fibrinolytic activity. Since epinephrine, atropine, and carbachol bring about swift changes in blood pressure which coincide with an increase in fibrinolytic activity, the question arises whether the rapid pressure change caused by the drugs is a common denominator for the activation of the fibrinolytic system or whether the change in blood pressure and the activation of the fibrinolytic system are concomitant but independent. So far it has not been possible to separate the phenomena in man or dog. Induction of fibrinolytic activity by pharmacological agents was always accompanied by blood pressure changes. Pyrogens and pitressin, which induce marked fibrinolytic activity in man, cause only moderate changes of blood pressure (about 15 mm/Hg)(8,9). These compounds, however, alter primarily the tonus of the vessels of the skin (pyrogens : dilation; pitressin : constriction). Future studies will show whether drugs which change primarily the tonus of veins and capillaries are able to induce activation of the fibrinolytic system. Such studies are likely to shed more light on the mechanism of this activation. The dog might well be a suitable animal for investigation of the endogenous activation of the fibrinolytic system.

Summary. Intravenous injections of carbachol, epinephrine, and atropine in dogs are followed by a marked transitory increase in fibrinolytic activity of venous blood. This could be repeated on the same dog at hourly and daily intervals without abating the effect.

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Isolation and Characterization of a Neurotropic Agent (MHG Virus) From Adult Rats. (28914)

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A transmissible agent has been recovered from laboratory white rats, which by its host range, physical and chemical properties, appears to be distinct from any previously described agent. Preliminary investigations suggest that infection with this agent or an antigenically related agent occurs in human beings.

The isolation was made in the autumn of 1961, from 3 adult white rats of the Sprague-Dawley strain. The rats showed signs indicative of a central nervous system involvement, including circling, incoordination, tremors and torticollis. The animals were not visibly disturbed by sudden noises or when spun by the tail. No gross lesions were observed upon necropsy, and bacteriological examinations of the middle ear canal, cerebrospinal fluid and brain tissues were negative. No significant findings were obtained from parasitological and hematological examinations.

At necropsy, specimens of brain, lung, liver and intestines were collected for biological examination and prepared as 20% tissue suspensions. These suspensions were free of bacterial, leptospiral and pleuropneumonia-like organisms, on cultural examinations.

This colony of rats had been under close supervision for several months and no additions had been made prior to the outbreak of the disease. Information on the isolation of the agent, designated as MHG virus, some of its physical-chemical properties and preliminary serological data are presented here.

Materials and methods. Cell cultures, embryonated eggs and animals. Primary kidney cell cultures of mouse, pig, rat, cow, rabbit, horse, hamster, rhesus monkey and primary cell cultures of whole mouse embryo and whole rat embryo were prepared from trypsinized cell suspensions and were grown in Hanks' balanced salt solution, containing 10% calf serum and 0.5% lactalbumin hydrolysate. Primary cell cultures and other cell lines (L929, human embryonic diploid brain, HeLa and AV-3) were maintained in Eagle's minimum essential medium containing 3% calf serum, 100 units penicillin and 100 μg streptomycin per ml. Cortisone-treated cells were fed with a maintenance medium containing 100 μg hydrocortisone sodium succinate per ml, 24 hours prior to use. This medium was decanted prior to addition of viral inoculum.

The Walter Reed Bagg Swiss strain of mice, the Sprague-Dawley strain of rats, rabbits and hamsters were obtained from the Dept. of Laboratory Animals, Walter Reed Army Inst. of Research. Embryonated eggs were purchased from a commercial source.

Animal tissues were prepared as 20% suspensions in Hanks' balanced salt solution containing 1.5% phenol red base broth, 0.5% lactalbumin hydrolysate (PBLH), 1,000 units of penicillin and 1,000 μg streptomycin. The suspensions were centrifuged at 4,000 rpm for 30 minutes. The supernatant fluid was collected and stored at —70°C until used.

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