Depression of Nerve-mediated Smooth Muscle Contractions 

*In Vitro* by Plasma of an Anephric Rabbit and 

Uremic Patients

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**Abstract** Isolated hypogastric nerve-vas deferens preparations from 

guinea pigs were used in vitro to detect effects of plasma solutes from a 
nephrectomized rabbit or uremic patients on nerve-mediated contractions. 
Plasma collected from the subjects was ultrafiltered and applied directly 
to the tissue in an organ-bath at 34–36°C. Plasma solutes with molecular weights (M.W.) smaller than 500 depressed contractions, their 
potency being greater when derived from the anephric rabbit and from 
patients than from the healthy control. Plasma solutes with a M.W. of 
500–10,000 from the healthy rabbit augmented contractions, whereas 
those from the anephric rabbit depressed them. Human plasma solutes 
with a M.W. of 500–5,000 or 5,000–10,000 exhibited no effect except a 
slight depression by those with a M.W. of 500–5,000 from uremic patients. 
High M.W. plasma solutes (10,000–20,000 daltons) from the patients were 
also found to depress contractions more strongly than those from healthy 
men. It is concluded that uremic metabolites capable of depressing 
peripheral autonomic functions accumulate in the nephrectomized rabbit 
and in patients with renal failure, and the degree of depression varies 
with the difference in M.W. of the metabolites.

The uremic symptoms include disturbed functions of nervous systems and 
muscles (Tyler, 1968; Raskin and Fishman, 1976a, b). Physiological exami-
nations of uremic patients frequently reveal an electroencephalographic abnormality (Tyler, 1968), a depressed sensory or motor nerve conduction velocity (Nielsen, 1974; Tackmann et al., 1974; Bolton, 1976; Lang and Forsström, 
1977; Hansen and Ballantyne, 1978) and a lower resting membrane potential 
of muscle fibers (Cotton et al., 1979). In the search for uremic toxins causing 
these alterations, Man (1979) has recently shown in a study *in vitro* with frog sural 
nerves that plasma ultrafiltrate of uremic patients inhibits the amplitude of the 
action potential.

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The isolated hypogastric nerve-vas deferens preparation of the guinea pig, originally introduced by Huković (1961), has been widely used to investigate the actions of drugs (e.g., Bentley and Sabine, 1963; Birmingham and Wilson, 1963). We considered that this preparation also would be useful for determining whether blood plasma from an anephric rabbit and uremic patients contains the solutes which affect nerve-mediated smooth muscle contractions in vitro. Accordingly, the present study was designed to detect readily quantifiable alterations in the nerve-mediated smooth muscle contractions after direct application of fractionated plasma components into the perfusate. This study was also of interest, as several clinical investigations recently have revealed the occurrence of an autonomic dysfunction in the patients with chronic renal failure (Nies et al., 1979; Rockel et al., 1979), but little study has been made in vitro on the effect of uremic plasma on peripheral autonomic nerves or effector organs. It will be shown that uremic plasma depresses the contractions more strongly than the healthy type and that the effectiveness of the plasma solutes varies with the difference in molecular weights (M.W.).

METHODS

Preparation of blood plasma solutes from rabbit and human subjects. Bilateral nephrectomy was performed on a male rabbit weighing 3.5 kg, under intravenous anesthesia with sodium pentobarbital (35 mg/kg). Forty-eight hr after the operation, 80 ml of blood was collected from a cannulated carotid artery of the rabbit under ether anesthesia. Before the collection the rabbit was heparinized (60 units/kg), and 80 ml of physiological saline solution was infused via the femoral vein during the collection. Assuming that the infused physiological saline solution diffused immediately throughout the circulating blood, the solutes in the collected blood were estimated to be diluted to approximately 0.8 of the original concentration in the body. For the sake of comparison, blood was collected in the same way from another healthy rabbit (male, 3.7 kg) that had received anesthesia with sodium pentobarbital (35 mg/kg) 48 hr before, but had no further surgical treatment. Forty ml samples of blood plasma were obtained from the anephric and healthy rabbits by centrifugation of the collected blood at 1,500 rpm for 30 min under cooling.

Human blood samples were taken from the medial cubital veins of the uremic patients admitted to Kumamoto Central Hospital. The patients consisted of 4 males and 3 females aged from 19 to 50 years; informed consent was obtained for this study. All patients had severe chronic renal failure without other concurrent serious illness, but had not yet received hemodialytic treatment. A total of 105 ml of blood samples was obtained from the 7 patients (15 ml each), from which 56 ml of plasma was obtained by centrifugation at 1,500 rpm for 30 min under cooling. For comparison, 30 ml of plasma was similarly obtained from...
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80 ml of blood samples from healthy males (n=2, 36-37 years old).

Ultrafiltration membranes graded in terms of M.W. cut-off levels were used for the concentration and further separation of the plasma from the anephric or healthy rabbit. Utilizing diaflo membranes of type UM05 and UM10 (Amicon Corp.), each plasma sample could be divided roughly into at least 3 fractions containing the solutes with a M.W. smaller than 500, 500-10,000, and more than 10,000, respectively. The last sample was discarded in the present study. Similarly, using the ultrafiltration techniques, the plasma from uremic patients or healthy men was divided into 5 fractions, each containing solutes with a M.W. less than 500, 500-5,000, 5,000-10,000, 10,000-20,000, and more than 20,000, respectively. The last sample was also discarded in the present study.

Hypogastric nerve-vas deferens preparation of guinea pig. Isolated hypogastric nerve-vas deferens preparations of guinea pigs (150-240 g) were mounted in an organ bath of about 5 ml capacity and perfused continuously with fresh Krebs solution at a rate of 150-200 ml per hr, as described previously (Juang et al., 1980). The temperature was kept constant at 34-36°C. Nerve-mediated contractions of vas deferens were produced by 1-sec tetanic stimulations (30 Hz, 0.2 msec pulse width) which were repeatedly delivered through platinum ring electrodes to the hypogastric (preganglionic) nerves at 2-min intervals in each experiment. The maximal stimulus intensity was used for each preparation. The ultrafiltered plasma solutes from each subject were directly added to the perfusate of the organ bath (while perfusion with fresh solution was stopped for 5 min) to examine the effects on nerve-mediated smooth muscle contractions. The contractions were isotonically recorded with a force-displacement transducer (RP-2, Nihon Kohden).

RESULTS

Although no spontaneous contractions of the vas deferens were induced in vitro by application of plasma solutes of the anephric and healthy rabbits or the uremic and healthy human subjects, the nerve-mediated contractions were increased or decreased, as described below.

In control experiments, in which Krebs solution was applied to the bath, the nerve-mediated contractions always showed a smooth and monophasic time course (e.g., Fig. 1A or C). Application of healthy rabbit plasma solutes with a M.W. of 500-10,000 at a high concentration produced a multi-contraction complex with augmented amplitudes and prolonged duration (Fig. 1B). Duration of these contractions in response to 1-sec tetanic stimulation lasted about 15 sec, which was 2-3 times longer than with the control. On the other hand, anephric rabbit plasma solutes with a M.W. of 500-10,000, as well as the other plasma fractions from the anephric and healthy rabbits and from the uremic and healthy human subjects, depressed the contractions without changing the time course.
Fig. 1. Examples showing time courses of guinea pig vas deferens contractions in response to hypogastric nerve stimulation. A and C, controls (before application of plasma solutes). B, 4 min after application of healthy rabbit plasma solutes with a M.W. of 500–10,000 at a concentration similar to the original concentration in the body. C, 6 min after application of anephric rabbit plasma solutes with a M.W. of 500–10,000 at a concentration similar to the original concentration in the body. Calibration, 1.0 g and 5 sec.

Fig. 2. Examples showing sequential changes in the amplitudes of guinea pig vas deferens contractions in response to hypogastric nerve stimulation. The stimulation was delivered to the vas at 2-min intervals. □, control experiment (n=8, mean±S.E.M.), in which 0.01–4.0 ml of Krebs solution was added to the perfusate in the organ bath. ○, application of healthy rabbit plasma solutes with a M.W. of 500–10,000 at a concentration similar to the original concentration in the body (n=1). ●, application of anephric rabbit plasma solutes with a M.W. of 500–10,000 at a concentration similar to the original concentration in the body (n=1). In each experiment, amplitudes of contractions are expressed as percentage changes from those obtained just before application of Krebs solution or plasma solutes. Time for the application is indicated by a horizontal bar with upward and downward arrows.

Sequential changes in the amplitudes of contractions before, during, and after application of rabbit plasma solutes with a M.W. of 500–10,000 are illustrated in Fig. 2. In the control experiments, the amplitudes of contractions were found...
to diminish gradually. The amplitudes decreased with some fluctuations at a rate of 21±8% (mean±S.E.M., n=8) per hr. Therefore, in order to assess the effectiveness of plasma solutes on the amplitudes, data obtained during and after application of plasma solutes were compared with those data at each corresponding time in the control experiments (see Fig. 3 below).

Healthy rabbit plasma solutes with a M.W. of 500–10,000 appreciably augmented the amplitudes within 4 min after application. In contrast, anephric rabbit plasma solutes with the same M.W. gradually decreased the amplitudes. These augmented or depressed contractions recovered to the control level within a relatively short period during the wash-out stage. Similar sequential changes in the amplitudes were also observed after application of the other plasma fractions from rabbit and human subjects.

Figure 3 shows dose-response curves obtained for effects of plasma solutes in vitro from rabbit and human subjects on the amplitudes of nerve-mediated smooth muscle contractions. Application of the rabbit plasma solutes with a M.W. less than 500 produced a dose-related depression of the amplitudes (Fig. 3A). At the highest concentration (0.8× the original concentration in the body)
tested, for example, the plasma solutes from anephric and healthy rabbit depressed the contractions to 3 and 66% of the control, respectively. Thus, the degree of depression was greater in the anephric rabbit than in the healthy one. On the other hand, the plasma solutes with a M.W. of 500-10,000 showed opposite effects between anephric and healthy rabbit (Fig. 3B), i.e., the solutes from the former depressed the contractions to 66% of that of the control, whereas the solutes from the latter augmented the contractions up to 116% of the control when tested at a high concentration (1.0× the original concentration).

The human plasma solutes with a M.W. less than 500 also showed dose-related depressive effects on the contractions (Fig. 3C). Similar to the results observed in rabbits (Fig. 3A), it was noticed that the depressive effect was greater with uremic solutes than with healthy ones. As compared with the rabbit plasma solutes of the same M.W., however, the effectiveness of the human plasma solutes was lower. The healthy human plasma solutes with a M.W. of 500-5,000 showed no effect, but the uremic ones depressed the contractions to 76% of the control when tested at a high concentration (1.0× the original concentration, Fig. 3D). Plasma solutes with a M.W. of 5,000-10,000 from neither the healthy men nor the uremic patients affected the contractions (Fig. 3E). On the other hand, plasma solutes with a M.W. of 10,000-20,000 either from healthy men or from uremic patients depressed the contractions in a dose-related manner, the effectiveness being greater in the uremic solutes than in the healthy ones (Fig. 3F). It was also noted that the depressions produced by human plasma solutes with a M.W. of 10,000-20,000 were greater than those by the low M.W. solutes (cf. Fig. 3C with Fig. 3F).

**DISCUSSION**

Additional studies are obviously necessary to examine the consistency of the present observations. Nevertheless, we have tentatively concluded that the uremic plasma contained biologically active substances which depressed nerve-mediated smooth muscle contractions *in vitro*. This is based on the following facts: (1) the depressing effect of the plasma fractions always showed dependency on the dose and the effectiveness was greater in the uremic plasma than in the healthy control (Fig. 3); (2) the effects of the plasma fractions subsided after wash-out of the tissues (Fig. 2); and (3) consistent changes in the contractions occurred when application of a plasma fraction was repeated, as demonstrated in our experiment (Fig. 3C).

Depression of the contractions, though to a smaller extent, was also observed in some cases of application of plasma fractions from a healthy rabbit and men (Fig. 3). Therefore, we cannot readily determine from the present data whether the depression by the uremic plasma fractions was caused by excessive accumulation of metabolites which were also present in the healthy animal and men, or...
whether the depression was caused by particular uremic metabolites which were present only in the uremic plasma. It may be stated, however, that the plasma from an anephric rabbit contained uremic metabolites which were absent in the healthy rabbit plasma, since the uremic plasma solutes with a M.W. of 500–10,000 depressed contractions, whereas the healthy ones with the same M.W. augmented them (Fig. 3B). Alternatively, the healthy rabbit plasma might contain particular substances capable of augmenting contractions, which were absent in the healthy human plasma (Fig. 3D and E).

The depression of the nerve-mediated smooth muscle contractions after application of the uremic plasma fractions could be due to one or more of the following: (1) initiation and propagation of impulses in the nerve fibers had deteriorated; (2) excitability of smooth muscle membranes was lowered, and/or excitation-contraction coupling systems in the smooth muscles were impaired; or (3) synaptic transmissions at the sympathetic ganglia and/or at the sympathetic neuromuscular junctions were disturbed. The first explanation is supported by the clinical observations such as a depressed sensory or motor nerve conduction velocity in the patients with chronic renal failure (NIELSEN, 1974; TACKMANN et al., 1974; BOLTON, 1976; LANG and FORSSTRÖM, 1977; HANSEN and BALLANTYNE, 1978) as well as by the experimental observation in vitro made by MAN (1979, see DISCUSSION below). The second explanation may also be possible, since COTTON et al. (1979) reported that resting membrane potentials of skeletal muscle fibers were lower in the patients with chronic renal failure. There is neither experimental evidence nor clinical observation to support the third possibility.

In the search for uremic toxins, BABB et al. in 1972 proposed the "middle molecule" hypothesis that the toxic products playing an important role particularly in the generation of peripheral neuropathy had a M.W. in the range of 500–5,000 daltons. MAN (1979) has recently reported that a middle molecular fraction of plasma ultrafiltrate from polyneuropathic patients inhibits the action potential amplitude of frog sural nerves in vitro. In the present study, the anephric rabbit plasma solutes with a M.W. of 500–10,000 (Figs. 1–2, and Fig. 3B), as well as the uremic patient plasma solutes with a M.W. of 500–5,000 (Fig. 3D), were found to depress contractions of the smooth muscle in response to nerve stimulation. We consider that the results of the present study are in line with the previous findings by MAN (1979), although development of neuropathy has not been examined in the present study. The origin of the effect of middle molecular fraction of the uremic plasma may be related to its depressing action on the sodium transport system, as found in epithelial cells of frog skin (BOURGOIGNIE et al., 1971) and on Na-K ATPase activities of the membranes (YAMADA et al., 1976), resulting in the accumulation of sodium ions in nerves and muscles, leading to lowered electrical activities.

Another candidate recently proposed for "neurotoxin" is parathyroid hormone (AVRAM et al., 1978; GOLDSTEIN et al., 1978), and its M.W. is known to be about
7,000 daltons. Our data do not support this, since the uremic patient plasma solutes with a M.W. of 5,000–10,000 did not produce any effect on the nerve-mediated contractions in the present study (Fig. 3E). However, our experiment is not conclusive, and should be repeated at higher concentrations using more finely fractionated plasma components of neuropathic patients. The uremic patient plasma solutes with a M.W. of 10,000–20,000 also depressed the nerve-mediated contractions to a large extent (Fig. 3F). In connection with the search for the uremic toxins, this observation is worthy of more investigation, since there is no report extant for the uremic toxins whose M.W. are in such a large range. On the other hand, numerous substances with a low M.W. (less than 500 daltons) are known to accumulate in patients with renal failure and characterize the uremic state. Methylguanidine (MG) is one such substance and is suspected to be a uremic toxin (GIOVANNETTI et al., 1973). It is unlikely, however, that MG was involved in the depressive effect of the low M.W. plasma solutes on contractions (Fig. 3A and C), since direct application of MG alone to the tissue in vitro was found to produce, rather, an augmentation of contractions (Matsumoto and Yonemura, unpublished observation). BOÉTHIUS and RYDQVIST (1977) have shown that urea disturbs the generation of action potentials in the frog muscle membranes. Thus, the depressive effect of the low M.W. plasma solutes observed in the present study might be attributable to urea to some extent.

All the effects of plasma solutes observed in the present study (Fig. 3) were transient and reversible by washing out the solutes from the tissue with a fresh Krebs solution. This may be related to the clinical observations that nervous dysfunctions in the patients with renal failure were frequently improved after successful renal transplantation or adequate hemodialysis (BOLTON, 1976; LANG and FORSSSTRÖM, 1977; RÖCKEL et al., 1979; LEWIS et al., 1980).

In conclusion, the present study demonstrates that plasma from an anephric rabbit and uremic patients contains the solutes which are capable of depressing the nerve-mediated smooth muscle contractions of guinea pigs in vitro. The degree of depression by the solutes varies with the difference in the M.W. of the solutes. Further studies with more finely fractionated plasma components are required for identification of the uremic toxins.

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