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Neural Influences on Bullfrog Renal Functions (40963)

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Abstract. The present investigations suggest that α-adrenergic mechanisms may be involved in the control of glomerular and tubular functions of bullfrog kidneys. Hypothalamic lesions did not appreciably affect the antidiuretic response of bullfrogs to dehydration. Phenoxybenzamine reversed the antidiuresis in dehydrated intact bullfrogs. We also performed experiments in which bullfrog kidneys were perfused through the dorsal aorta and renal portal veins *in situ*. In such perfused kidneys, the renal portal perfusate did not reach the glomeruli. Phenoxybenzamine applied to the arterial perfusate blocked the antidiuretic effect of norepinephrine and produced diuresis and increased renal perfusion. The same response was observed with pithing or destruction of the spinal cord and central nervous system. Phenoxybenzamine given through the left renal portal perfusion would reach the tubules of the left kidney. It produced diuresis and natriuresis in the treated kidney but not the right control kidney. We conclude that neural activities influence preglomerular vasoconstriction and tubular reabsorption in bullfrogs.

Recent studies have shown that in addition to the previously established endocrine controlling mechanisms, neural influences on renal functions contribute to the regulation of the mammalian kidney. The innervation of the afferent and efferent arterioles of the glomeruli and other renal vasculature can affect renal blood flow and distribution which can in turn influence the glomerular filtration rate and other aspects of renal function (1). In addition, tubular cell innervation has been demonstrated morphologically (2) and its effect on tubular function has also been described (3, 4).

There is almost no information concerning the neural control of renal function in lower vertebrates. In 1869, Beale (5) described the presence of nerve fibers in the vicinity of amphibian renal capillaries and tubules. This finding has been repeatedly substantiated by subsequent investigators (6–8). However, there have been few studies of the functional significance of these morphological findings. Richards and Walker (9) and Forster (10) reported that catecholamines are capable of constricting the afferent glomerular arterioles and reducing the glomerular filtration rate in amphibian kidneys.

In some recent studies we measured the circulating levels of arginine vasotocin (AVT), the antidiuretic hormone, in bullfrogs during water deprivation and observed no measurable change in some frogs although maximal antidiuresis was evident in these animals. In addition, hypertonic saline infusion also produced antidiuresis without a detectable change in circulating levels of AVT. We suspected that some other controlling mechanisms were playing an important role in causing antidiuresis in these bullfrogs (11). The present report describes a series of experiments on the importance of α-adrenergic innervation in the control of glomerular and tubular activities of amphibian kidneys.

Materials and Methods. Adult bullfrogs, *Rana catesbeiana,* of both sexes were purchased from a commercial supplier in Louisiana and kept in the laboratory in standing dechlorinated tap water at room temperature for at least 1 week before use in experiments. The body weights of the animals were between 250 and 600 g. Two

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main types of experiments were performed. In one type of experiment, the blood pressure and urine volume were determined in conscious frogs. In another group of experiments, renal functions were investigated in perfused kidneys.

The analytical methods were as follows. Blood pressure was determined with a Statham pressure transducer and recorded on a Grass polygraph. Sodium levels were analyzed with an Instrumentation Laboratory flame photometer. Creatinine and inulin were assayed by the alkaline picrate and acid resorcinol methods, respectively (12).

(1) *In vivo experiments.* Bullfrogs were anesthetized with tricaine methanesulfonate (Finquel, Ayerst) and cannulated according to the method of Uranga and Sawyer (13). The two ureters were cannulated with PE 90 tubing using a dorsal approach. The iliac artery and the musculocutaneous vein were also cannulated to allow arterial blood pressure recording and intravenous administration of drugs. The experimental animals were housed in plastic boxes covered with aluminum foil because our experience has shown that bullfrogs are very sensitive to the movement of objects around them and that slight visual or auditory disturbances will alter blood pressure and urine flow. The bullfrogs were left in the boxes for at least 24 hr before use in experiments. In water deprivation experiments, the water in the box was removed with minimal disturbance to the frogs through rubber tubing connected to the bottom of the box.

Two kinds of *in vivo* experiments were performed. In the first, hypothalamic lesions were made on bullfrogs according to the methods of previous investigators (14–16). The controls were mock-operated. Experiments did not begin until at least 1 week after the operation. Both the control and the experimental animals were dehydrated by removing water from their boxes. Ureteral urine volumes were recorded for each animal daily for 2 days before and 4 days after water deprivation. There were seven animals in each group.

Cannulated intact frogs were used in the second series of *in vivo* experiments. Average arterial blood pressure, urine volume and urine sodium excretion were determined for every 15-min period during the experiment. Four determinations were made before water deprivation. Fifteen hours later, the animals were studied for 2 hr. Intravenous injection of phenoxybenzamine (Smith, Kline and French) at the dose of 10 mg/kg body wt was then given to the frogs and blood pressure and urine volume were followed for an additional 1½ hr. A total of seven animals were used in these studies.

(2) *In situ experiments.* Four series of *in situ* experiments were conducted. In these studies bullfrog kidneys were perfused with amphibian Ringer's solution. One liter of the solution contains 80 mmole NaCl, 2.5 mmole KCl, 1.8 mmole $CaCl_2$, 24 mmole $NaHCO_3$, 1.1 mmole glucose, 200 mg creatinine, 250 mg glycine, 10 g mannitol, 7.5 g dextran (average molecular weight 75,000), and 25,000 *U* heparin. This solution is aerated with 95% O_2 and 5% CO_2. The basic experimental setup is similar to that reported by Pang and Sawyer (17) for arterially perfused bullfrog kidneys. The bullfrog was anesthetized as in the *in vivo* experiments and the abdominal cavity was opened ventrally. The ureters were cannulated with PE 90 tubing to allow continuous ureteral urine collection. The dorsolumbar vein and celiacomesenteric artery were ligated. The postcaval vein was tied off between the kidneys and liver and was cannulated to drain the perfusion fluid from the kidneys. The rate of perfusion through the kidneys can be determined by collecting the fluid from this cannula with a graduated cylinder. The mesenteric and other branches of the dorsal aorta anterior to the kidney were ligated. The dorsal aorta just anterior to the urogenital artery was connected to a perfusion chamber (a 20-ml syringe barrel). The level of the amphibian Ringer's solution in the perfusion chamber was kept constant by two stainless-steel electrodes connected to the chamber through Ringer–agar bridges. These two electrodes were joined to a relay which controlled a peristaltic pump delivering aerated Ringer's solution from a reservoir to the perfusion chamber. When the level of

the fluid in the perfusion chamber fell below that of the electrodes, the pump was turned on. Thus, the perfusion pressure was kept rather constant, within a few millimeters of water. The left and right renal portal veins were also perfused with setups similar to that described for the arterial perfusion. In this way the kidneys were perfused through the three major normal routes of blood supply. The entire setup is shown in Fig. 1.

This experimental approach has many advantages. The renal portal circulation irrigates the renal tubules but not the glomeruli. By comparing the effects of drugs and chemicals administered to the renal portal perfusion and the arterial perfusion one can differentiate between their tubular and glomerular effects. However, if the perfusion pressure is excessive, the renal portal perfusate may reach the glomeruli. To avoid this, the arterial perfusion pressure was maintained between 25 and 30 cm water and the perfusion from the renal portal cannulas, around 8 cm water. In each experiment, the system was tested prior to the administration of any drug to make sure that the renal perfusion did not reach the glomeruli. Inulin and phenol red were both added to the renal portal perfusate. The appearance of phenol red, but not inulin, in the urine indicates that the renal portal perfusate did not come into contact with the glomerular circulation but did reach the renal tubules. In each experiment, inulin determinations were made for the initial urine samples. If inulin appeared in the urine, the perfusion pressures were adjusted. When no inulin appeared in the urine, the experiment was begun. At the end of each experiment, all urine samples were tested for the presence of inulin. In all experiments reported here, inulin was not observed in any urine samples.

Drugs were injected directly into the arterial or the venous cannulas. Substances given arterially reach the glomeruli and the effects on the pre- and postglomerular circulation are reflected in the flow rates from the kidney and in urine volume. Since the arterial perfusate eventually reaches the renal tubules, the effects could also be tubular in origin. However, since substances administered through the venous perfusate reach the renal tubules but not the

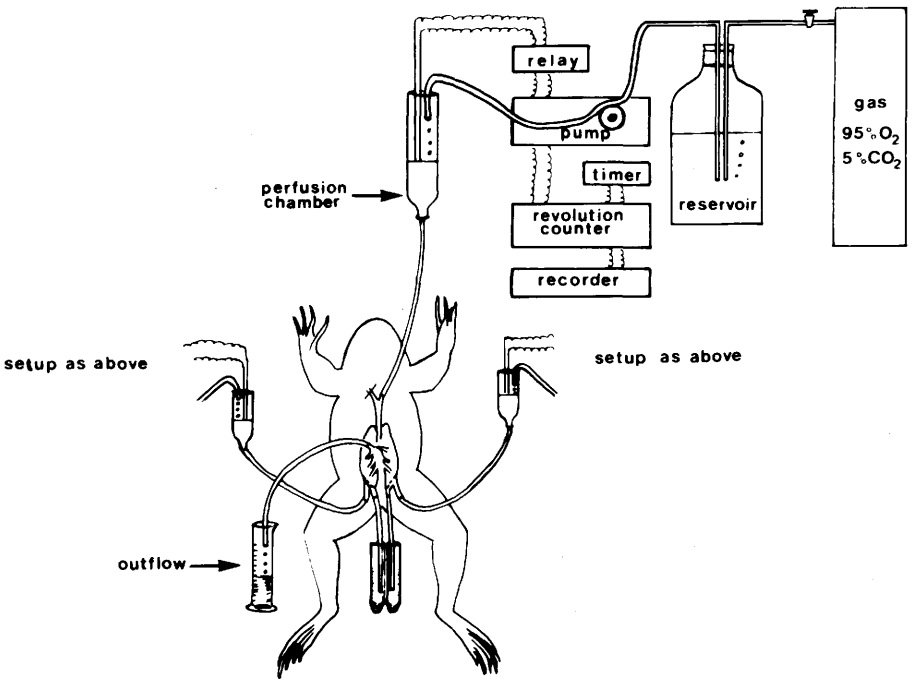

FIG. 1. The experimental setup of *in situ* bullfrog double-kidney perfusion.

glomeruli, their tubular effects can be determined. Such an assumption was verified by the absence of inulin in the urine and the absence of changes in perfusion rates. Analysis of data from arterial and venous drug administration together allows us to distinguish tubular from glomerular actions of a drug. Furthermore, since the renal portal veins are perfused separately and the urine is also collected separately from the two kidneys, one kidney can serve as the control for the other when the action of various drugs are tested by injection into one portal vein.

Four types of experiments were performed on perfused bullfrog kidneys. In the first, the effects of pithing on urine volume and outflow rate were studied. In the second, the effects of norepinephrine (Sigma) (10 μg/kg) on urine volume, creatinine clearance, and outflow rate were determined in pithed frogs. In the third, the kidneys were perfused arterially with phenoxybenzamine at the concentration of 20 μg/ml and the effects on urine volume and outflow rates were recorded in unpithed frogs. While under treatment with the adrenergic blocker, norepinephrine at the doses of 10 and 50 μg/kg and AVT (MM ⑦236, kindly supplied by Dr. Maurice Manning) at the dose of 10 ng/kg, were tested. In the fourth experiment, the left kidney was perfused with phenoxybenzamine (20 μg/ml of Ringer's solution) through the renal portal vein in unpithed frogs while the right kidney served as the control. In this experiment, urine volume, creatinine clearance, and urine sodium excretion from each kidney and the total outflow rate from both kidneys were determined.

Results. Hypothalamic lesions did not affect the ability of water-deprived, intact bullfrogs to reduce urine volume as compared to operated control animals (Fig. 2). The onset and the degree of antidiuresis were similar in both groups. In normal intact frogs, water deprivation resulted, as expected, in antidiuresis with a decrease in blood pressure. Phenoxybenzamine, an α-adrenergic blocking agent, increased the urine volume and urinary sodium excretion but decreased the arterial blood pressure. The urine volume was similar to that of the

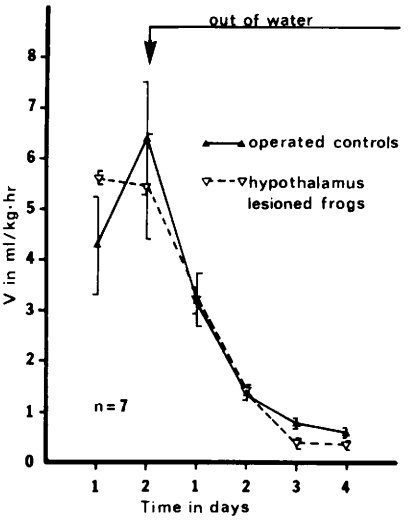

FIG. 2. Effects of dehydration on hypothalamus-lesioned and mock-operated bullfrogs. V = urine volume.

period before water deprivation. These results are shown in Fig. 3.

In the first perfused kidney experiment, pithing increased both the urine volume and renal perfusion rate as indicated by the renal venous outflow of the perfusion fluid (Fig. 4). This suggests that an intact central nervous system and spinal cord are necessary for maintaining renal vascular tone and urine volume in these perfused kidneys. When the pithed frogs were given norepinephrine, antidiuresis with a reduction in creatinine clearance and renal perfusion rate were observed. These results are summarized in Fig. 5. When the kidneys were perfused in frogs with an intact nervous system, arterial administration of phenoxybenzamine produced diuresis and an increase in renal perfusion rate. Administration of norepinephrine under such circumstances had little or no effect, but AVT produced antidiuresis and a reduction in renal perfusion rate. These data are shown in Fig. 6. They indicate that while the adrenergic antagonist blocked the action of norepinephrine, the kidneys were still capable of responding to another agent, AVT, which is known to produce antidiuresis in the bullfrog. In the unpithed frogs, renal portal venous administration of phenoxybenzamine elicited diuresis and natriuresis in the left, but not

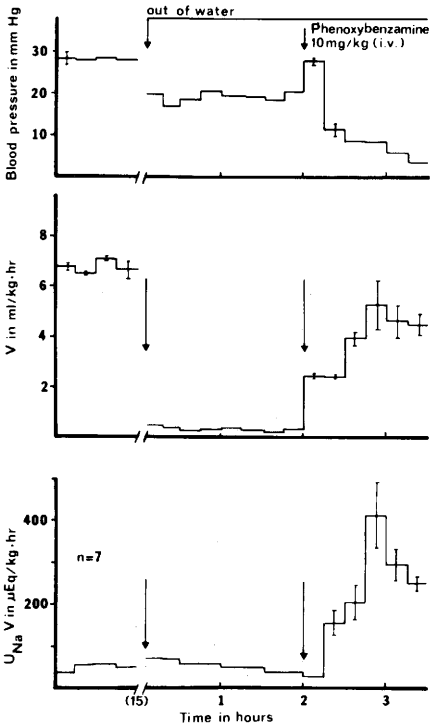

FIG. 3. Effects of phenoxybenzamine on blood pressure, urine volume (V), and sodium excretion ($U_{Na}V$) of dehydrated bullfrogs.

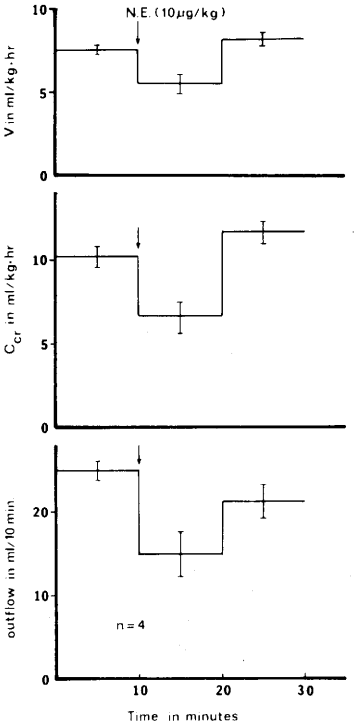

FIG. 5. Effects of norepinephrine on urine volume (V), creatinine clearance (C_{Cr}), and renal perfusion outflow rate in perfused kidneys of pithed bullfrogs. The changes produced by norepinephrine are significant at $P < 0.05$.

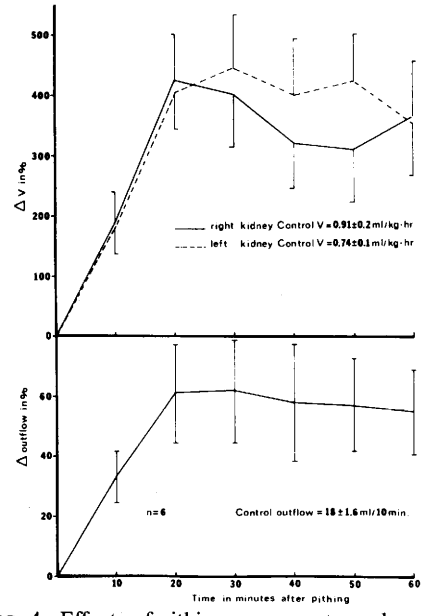

FIG. 4. Effects of pithing on percentage changes of urine volume (ΔV) and renal perfusion outflow rate (Δoutflow) in perfused bullfrog kidneys *in situ*.

the right control kidney in the same animal. There was no significant change in the renal perfusion rate. Creatinine clearance tended to rise during phenoxybenzamine treatment but returned to initial levels after 1 hr while urine flow remained elevated (Fig. 7). There is no correlation between changes in creatinine clearance and urine flow rates.

Discussion. The results of the present series of experiments suggest that the bullfrog nervous system is involved in the regulation of kidney functions. Although AVT is an antidiuretic hormone in the lower tetrapods, the destruction of the hypothalamus, the site of AVT production, did not prevent bullfrogs from responding to water deprivation with antidiuresis. It has also been reported that hypothalamic lesions in toads did not abolish the antidiuretic response to dehydration (14, 16). Thus mechanisms other than AVT release can produce antidiuresis. Neural input to the kidneys may be one of these.

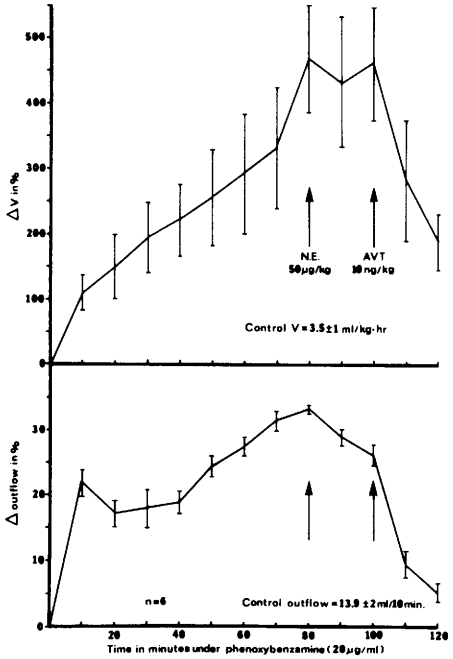

FIG. 6. Effects of phenoxybenzamine on perfused bullfrog kidneys *in situ*. The effects of norepinephrine (N.E.) and arginine vasotocin (AVT) were tested on the treated kidneys.

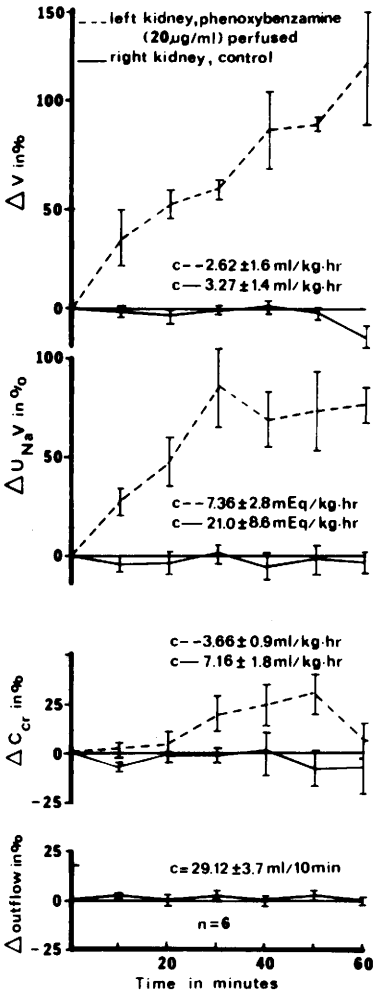

FIG. 7. Effects of phenoxybenzamine on urine volume, sodium excretion, creatinine clearance, and renal perfusion outflow rate of perfused bullfrog kidneys. The drug was given to the left kidneys. The right kidneys served as controls. *c* = preinjection control values.

In the first perfused kidney experiment, pithing resulted in diuresis and an increase in renal perfusion. This would suggest that there is significant neural control of the preglomerular circulation. Neural activity would tend to constrict the preglomerular arterioles while termination of the neural input would relax the arterioles and increase urine production by increasing flow and pressure in the glomerular capillaries. This is consistent with the effect of phenoxybenzamine in the *in vivo* studies. Our hypothesis is further substantiated by the effect of norepinephrine on the kidneys of pithed frog. Since this catecholamine was administered arterially, it would, therefore, reach the glomerular circulation as well as the renal tubules. The reduction of urine volume, glomerular filtration rate, and renal perfusion rate suggests that the adrenergic agonist produced vasoconstriction in the preglomerular arterioles.

In unpithed frogs, phenoxybenzamine, an α-adrenergic blocking agent, produced effects similar to those of pithing and opposite to those of norepinephrine administration. We know that the antagonist blocked the receptors since administration of exogenous norepinephrine to bullfrogs treated with phenoxybenzamine failed to produce any effect. We showed, however, that the kidneys were still functioning since AVT produced antidiuresis and a reduction in renal perfusion. We concluded that α-adrenergic receptors are involved in the neural control of preglomerular vasoconstriction in the bullfrog. This hypothe-

sis is in agreeement with the findings of Richards and Walker (9) and Forster (10) who reported that catecholamines were capable of constricting the afferent glomerular arterioles and reducing glomerular filtration rate in amphibian kidneys.

Although we can draw some conclusions concerning the neural control of the preglomerular vasculature, the above data do not give us any indication of the neural control of renal tubular function. We have shown that drugs administered through the renal portal perfusate will reach the renal tubules but not the glomeruli since inulin given in the portal circulation did not appear in urine and phenoxybenzamine did not change perfusion rate when given portally. This would, then, be a logical approach to demonstrate the neural control of renal tubular function. When phenoxybenzamine was added to the left renal portal perfusion, diuresis and natriuresis were evident while values for the right kidney, which served as a control in the same animal, remained constant. This suggests a neural influence on renal tubular reabsorption. Normally, the neural activity would promote tubular reabsorption. Blockade with phenoxybenzamine reduced tubular reabsorption and hence the frog exhibited diuresis and natriuresis. This cannot be explained by the effect of the drug on the glomeruli alone since there are three experimental periods when extreme diuresis was observed without an increase in glomerular filtration rate. There are, however, some periods during which the glomerular filtration rates were elevated. We are not sure of the reasons for this elevation. The renal outflow remained unchanged during the experiment. In similar preliminary experiments on pithed frogs, phenoxybenzamine failed to elicit diuresis (Gallardo, Pang, and Sawyer, unpublished data).

In some recent morphological studies on the bullfrog kidney, we observed neural elements in close proximity to glomerular vessels and renal tubules with both light and electron microscopic techniques (Gallardo, Yee, and Pang, unpublished data). The presence of such innervation is consistent with our evidence that neural input can participate in the regulation of glomerular and tubular functions.

We do not, at present, know the exact mechanism of these neural influences. Do the nerves act directly on the renal elements or indirectly through the release of some other substances? It is most unlikely that the renin–angiotensin system is involved, since in the perfused kidney studies, the perfusing fluid contains no renin substrate and was not recirculated. Under these conditions it would be difficult to envisage how the release of renin could produce an effect on the preglomerular circulation. However, the participation of other locally released substances such as prostaglandins cannot, at this time, be ruled out.

Since the renal portal system irrigates both the proximal and distal portions of the renal tubules, our data did not give us any clue as to the exact site(s) of the action of the neural influence. Micropuncture studies would be useful in solving this problem.

The present studies indicate that our perfused bullfrog kidney setup provides a useful experimental approach to demonstrate the glomerular and tubular actions of drugs and hormones. The presence of the renal portal system enables investigators to observe the effects of drugs on the renal tubules without the complication of glomerular involvement. We have used this system to study the renal action of neurohypophyseal hormones and angiotensin (17–19). This type of perfused kidney system has also been recognized and utilized by other renal physiologists to study renal electrolyte transport (20).

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