Thus pyracetam reduces $K^+$-stimulated $^3H$-D-aspartic acid release from rat cerebral synaptosomes, playing the role of agonist of glutamate (aspartate) autoreceptors of quisqualate subtype, and this may be of significant importance for manifestation of the antihypoxic action of the compound. Pyracetam can also abolish the proline-induced intensificatin of D-aspartic acid release by a mechanism unconnected with quisqualate receptors, evidence of the existence of functional antagonism between these two substances, with their opposite action on memory processes.

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# PHARMACOLOGIC PREVENTION OF CISPLATIN NEPHROTOXICITY IN RATS


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Cisplatin is a new and effective chemotherapeutic agent used in the treatment of malignant tumors [1, 3], but it often disturbs renal function [7]. In order to prevent the nephrotoxic action of the preparation various methods are used to increase diuresis [3, 6, 7], and these have alleviated but not prevented kidney damage. Since administration of cisplatin causes severe damage to cells of the straight part of the proximal tubule of the nephron [5], an important role in the mechanism of production of renal failure could be played by its entry into those cells of the tubule that are able to secrete organic substances. In this case pharmacologic screening of the secretory apparatus during the peak period of cisplatin excretion would give protection against renal failure. The investigation described below was undertaken to test this hypothesis.

## EXPERIMENTAL METHOD

Experiments were carried out on 164 albino rats of both sexes weighing from 75 to 250 g. Cisplatin (DDP) was injected intraperitoneally in a dose of 0.5 mg/100 g body weight. The state of the renal function was assessed by administering water to the rats by gastric tube in a dose of 5 ml/100 g body weight, the animals were placed in individual constraining cages, and the urine excreted during 2 h was collected. Sodium and potassium levels in the blood serum and urine were determined on a Flapho-4 flame photometer, calcium and


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TABLE 1. Effect of Furosemide, Ethacrynic Acid, and para-Aminohippurate (PAH) on Diuresis (in ml/100 g·h) and Concentration of Electrolytes (in μmoles/ml) and Urea (in mg/100 ml) in Rats' Blood Serum (M ± m)

| Experimental conditions | Number of animals | Diuresis after water loading (2 h) | Serum concentrations of | | | | |
|---|---|---|---|---|---|---|---|
| | | | urea | Na | K | Ca | Mg |
| Control | 8 | 1,59±0,09 | 33±7 | 138±1 | 4,9±0,2 | 2,6±0,1 | 1,3±0,1 |
| Cisplatin | 8 | 0,59±0,07 | 194±36 | 137±1 | 5,8±0,2 | 3,1±0,2 | 1,3±0,1 |
| Furosemide | | | | | | | |
| 40 min before | 8 | 1,42±0,19 | 79±20 | 133±2 | 5,0±0,2 | 2,7±0,1 | 1,3±0,1 |
| 3 h after | 8 | 2,13±0,37 | 98±36 | 133±2 | 4,2±0,2 | 2,4±0,1 | 1,4±0,1 |
| 6 h after | 8 | 0,57±0,22 | 204±32 | 129±1 | 4,1±0,1 | 2,5±0,1 | 1,3±0,1 |
| PAH – 40 min before | 8 | 0,91±0,17 | 91±17 | 134±1 | 6,3±0,6 | 2,8±0,2 | 1,4±0,1 |
| Ethacrynic acid –40 min before | 8 | 1,16±0,25 | 95±13 | 134±1 | 4,9±0,2 | 2,8±0,1 | 1,3±0,1 |

Legend. Investigations carried out 3 days after injection of cisplatin. Time of administration of diuretics and PAH indicated relative to injection of cisplatin.

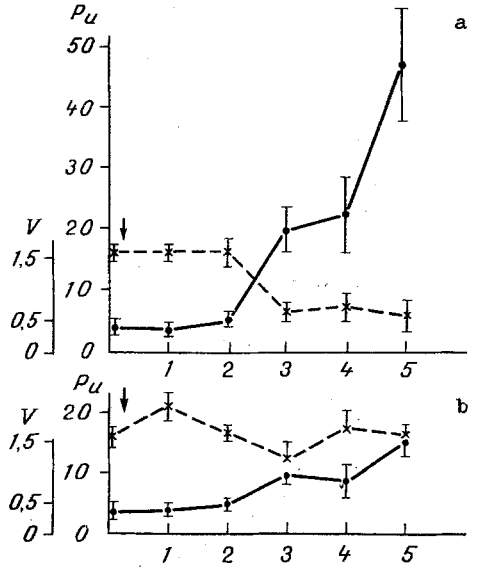


Fig. 1. Blood serum urea concentration (continuous line) and diuresis after water loading (broken line) in rats receiving ethacrynic acid and cisplatin. Abscissa, time of experiment (in days); ordinate, blood urea concentration ($P_u$, in μg/ml) and diuresis (D) during 2 h after water loading (in ml/h/100 g body weight). a) Intraperitoneal injection of cisplatin in a dose of 0.5 mg/100 g (arrow); b) administratin of ethacrynic acid by gastric tube in a dose of 0.5 mg/100 g and intraperitoneal injection of cisplatin 40 min later in a dose of 0.5 mg/100 g (arrow).

magnesium were determined on a Hitachi Model 508 atomic absorption spectrophotometer (Japan) and urea was determined with the aid of diacetomonoxime.

## EXPERIMENTAL RESULTS

The dominant feature of nephrotoxicity and development of acute renal failure is a raised blood urea level [3]. The blood urea concentration rose as early as 2 days after injection of cisplatin into the rats, on the 3rd day the increase was almost sevenfold, and on the 5th day 14-fold (Fig. 1). Administration of furosemide to the rats 30-40 min be-

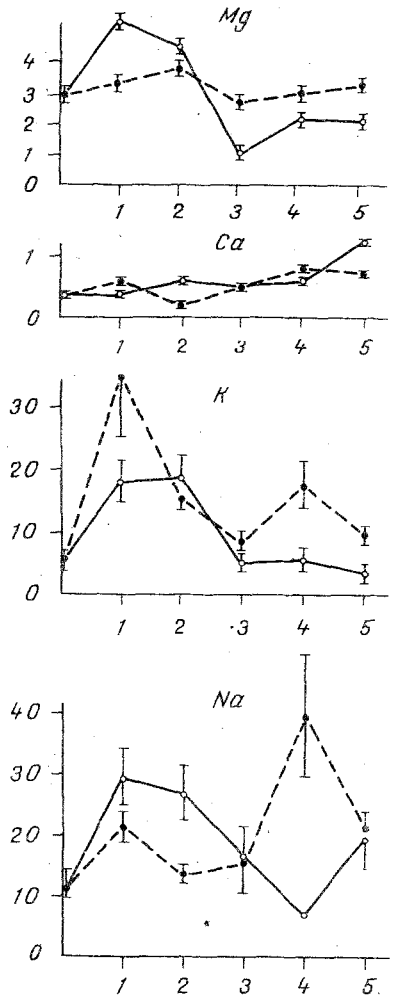


Fig. 2. Excretion of electrolytes by rat kidney after administration of ethacrynic acid and cisplatin. Abscissa, time of experiment (in days); ordinate, electrolyte excretion with (U·V) during 2 h after water loading (in μeq/h/100 g body weight). Broken line – intraperitoneal injection of cisplatin in a dose of 0.5 mg/100 g; continuous line – ethacrynic acid was given by gastric tube in a dose of 0.5 mg/100 g 40 min before injection of the same dose of cisplatin. Arrow indicates time of administration.

fore injection of cisplatin could give a prophylactic effect on account of two mechanisms: 1) furosemide is secreted in the proximal tubule and, reducing the entry of cisplatin into the cells of this tubule competitively, it can reduce nephrotoxicity; 2) furosemide increases diuresis, which causes the cisplatin concentration in the lumen of the tubule to fall. In fact, furosemide had a marked protective action and after 2 days the blood urea was lower than after injection of cisplatin alone (Table 1).

To test which of these mechanisms is involved in the therapeutic action of furosemide, in a similar experimental situation another diuretic was administered, namely ethacrynic acid (Uregyt, from Egyt, Hungary), which has marked ability to inhibit secretion of substances, including furosemide, in the proximal tubule [2], but, in experiments on rats, has no significant diuretic action. Administration of ethacrynic acid by gastric tube in a dose of 0.5 mg/100 g had a marked protective effect, comparable with that of furosemide (Fig. 1; Table 1). Screening cells of the proximal tubule of the nephron by administration of substances which competitively inhibit the secretion of organic acids and, perhaps, of other xenobiotics also, thus protects the animal against the nephrotoxic action of cisplatin.

Substances with no diuretic effect, but competitively reducing secretion of substances in the proximal tubule, could have a prophylactic action. Sodium para-aminohippurate (PAH) was injected into the rats in a dose of 4 mg/100 g 40 min before injection of cisplatin. Its therapeutic effect was distinctly observed and did not differ statistically significantly from that of furosemide (Table 1).

The nephrotoxicity of cisplatin thus depends largely on involvement of the secretory apparatus of cells of the proximal tubule, probably on account of accumulation of cisplatin in these cells during secretion induced by their injury. If this hypothesis is correct, the protective action of substances used to prever nephrotoxicity ought to depend on the time of administration. In special experiments furosemide was given before and 3 and 6 h after cisplatin. The results showed a protective action only if fuorsemide was administered before or during the first few hours after cisplatin. No prophylactic effect was observed when furosemide was given 6 h after cisplatin, and the blood urea on the 3rd day reached the same level as when cisplatin alone was injected (Table 1).

A single dose of furosemide, ethacrynic acid, or PAH, given before cisplatin, prevents the development of uremia, but nevertheless the blood urea concentration of these animals was higher than in the control. The explanation of this may be that these drugs protect the kidney during the most dangerous, first phase of excretion of cisplatin, and later, during gradual excretion of the residual cisplatin, the renal parenchyma undergoes partial damage. Two phases of excretin of cisplatin from the blood are known to occur: one with a half-period of 25-49 min, the second with a half-period of 58-73 min [4]. Investigation of renal function during the course of action of cisplatin was of great importance. Water loading experiments showed that during the development of renal failure induced by cisplatin excretion of fluid falls. This defect of kidney function, like the development of uremia, is abolished by administration of ethacrynic acid, PAH, or furosemide (Fig. 1). If, however, furosemide was injected 6 h after cisplatin, the disturbance of renal function was the same as in rats with developed uremia (Table 1). Close correlation was observed between the development of uremia and impairment of the ability of the kidney to excrete fluid after water loading (Fig. 1).

The study of serum levels of electrolytes (Table 1) and their excretion by the kidney (Fig. 2) and comparison of these results with the magnitude of diuresis after water loading (Fig. 1; Table 1) demonstrate that the nephrotoxic action of cisplatin causes no abrupt changes of electrolyte metabolism, but is manifested primarily as retention of urea in the body and impairment of the ability of the kidney to excrete fluid after water loading.

Preliminary or simultaneous administration of furosemide, ethacrynic acid, or PAH thus prevent to a considerable degree the nephrotoxic action of cisplatin and protect the ability of the kidney to excrete fluid after water loading. The mechanism of the prophylactic action of the substances tested is probably based on a decrease in the quantity of cisplatin entering the cells of the proximal tubule, which are able to secrete xenobiotics.

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