Pharmacodynamics of Phenobarbital Anesthesia and Pentylenetetrazol-Induced Maximal Seizures in a Rat Model of Neoplastic Spinal Cord Compression

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The purpose of this investigation was to determine whether paraplegia induced by neoplastic cord compression affects the pharmacodynamics of phenobarbital general anesthesia or of pentylenetetrazol (PTZ)-induced convulsions. Paraplegic rats harboring a thoracolumbar epidural tumor, or an identical hindlimb tumor mass, received an i.v. infusion of phenobarbital until the onset of anesthesia. At that point, the phenobarbital concentrations in the CSF and serum were measured. Similarly, PTZ was infused until the onset of maximal seizures. It was found that changes related to systemic tumor growth and newly developed paraplegia due to neoplastic spinal cord compression did not attenuate the pharmacodynamics of phenobarbital. However, sustained paraplegia of 4 days' duration reduced CNS sensitivity to the hypnotic action of the barbiturate as evidenced by the higher cerebrospinal fluid phenobarbital concentration required to induce anesthesia (170 ± 31 vs 125 ± 20 mg/L; $P < 0.05$). On the other hand, sustained paraplegia did not affect brain threshold concentration for PTZ-induced seizures.

KEY WORDS: pharmacodynamics; anesthesia; seizures; phenobarbital; pentylenetetrazol; spinal cord compression; paraplegia; tumor; concentration–effect relationship.

INTRODUCTION

Cancer patients are often treated with neuroactive agents, either for the control of pain or for the performance of invasive procedures that require the use of anesthesia or for subduing some side effects of chemotherapy. To optimize the doses of neuroactive medications, the influence of the basic disease on the kinetics of these drugs' action must be understood. However, such information is surprisingly limited. In particular, changes in the central nervous system's (CNS) sensitivity to the pharmacologic effects(s) of neuroactive drugs in the presence of systemic malignancy are not well documented. In the present investigation we used an experimental animal model of neoplastic spinal cord compression that resembles conditions frequently encountered in cancer patients. This model was selected because it combines both the effect of systemic tumor and the direct damage it induces on the spinal cord.

Spinal cord compression is a frequent oncologic emergency condition. Epidural spinal metastases are present in about 5% of cancer patients (1), and 20% of them will develop overt cord or cauda equina compression (2). Neurological symptoms include pain, muscle weakness, and bladder and bowel dysfunction, and, as the situation worsens, a complete loss of cord function, in the form of paraplegia and incontinence, takes place.

Because of the variability in the severity of the disease state and in the clinical presentation, it is appropriate to investigate its influence on pharmacodynamics under controlled experimental conditions using a well-characterized animal model. The model we used of neoplastic spinal cord compression was previously developed by Siegal and coworkers and was reported to induce various local changes in spinal cord biochemistry such as alterations in arachidonic acid metabolism (3), serotonin turnover (4), and levels of excitatory amino acids (5,6). These changes have an unknown effect on the sensitivity of the CNS to the pharmacological action of various neuroactive medications. The present investigation assesses the effect of neoplastic spinal cord compression on the pharmacodynamics of two well-established pharmacodynamic probes, one CNS depressant, phenobarbital (7), and an analeptic agent, pentylenetetrazol (PTZ). Whereas phenobarbital-induced anesthesia may be used as a model hypnotic drug, PTZ-induced seizures serve as a marker of neurotoxicity (8).

MATERIALS AND METHODS

To obtain clear information on the pharmacodynamic effects, while excluding interferences due to changes in the pharmacokinetics (i.e., concentration–time relationship) of phenobarbital and PTZ, the drugs were infused intravenously to the rats until a predefined pharmacological endpoint had been achieved: onset of anesthesia for the depressant drug or onset of maximal seizures for PTZ. At that point, the drug concentration at a sampling site which reflects the drug concentration at the site of action was determined. For the hypnotic action of phenobarbital the cerebrospinal fluid (CSF) is the appropriate sampling site (7), and for PTZ-induced maximal seizures, serum, brain, and CSF are equally suitable sites (9). The pharmacodynamic influence of the disease was determined by comparing the drug concentrations required to induce the same pharmacologic effect in diseased and control animals.

Animals and Tumors

Adult Fischer female (for phenobarbital experiment) or male (for PTZ experiment) rats were obtained from the Animal Breeding Center of the Hebrew University (Jerusalem, Israel). The tumor used in these experiments was a methylcholanthrene-induced malignant fibrous histiocytoma, maintained in our laboratory by serial subcutaneous transplantations in syngeneic rats. The tumor was removed after 3 to 4 weeks, minced with fine scissors in Roswell Park Memorial Institute (RPMI) medium (Grand Island Biological Co.,

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Grand Island, NY) under aseptic conditions, and treated with 0.05% trypsin–0.02% EDTA for 20 min at 37°C. The cell suspension was washed twice, centrifuged at 1200 rpm for 10 min, and assayed for viability by the trypan blue exclusion test. The cells were resuspended at an appropriate inoculum concentration in RPMI medium.

The rats were inoculated with 10^6 viable tumor cells in 0.1 mL of RPMI medium. The injection was carried out percutaneously under light ether anesthesia, anterolateral to T12 or T13 vertebral bodies. After inoculation, the animals were observed periodically as they developed neurological dysfunctions defined as hypotonic tail, hindlimb weakness, inability to stand, and finally, paraplegia (no voluntary hindlimb movements). Rats from the control group underwent the same procedure but were inoculated with normal saline.

To isolate the pharmacodynamic effects which evolve due to the presence of the tumor itself, tumor cells were also injected into the right hindlimb muscle of another group of rats.

Protocol of the Pharmacodynamic Experiment

One day before the pharmacodynamic experiment, an indwelling cannula was implanted in the right jugular vein, under light ether anesthesia, and filled with normal saline. The animals were housed individually in metal cages, with water and food available ad libitum.

Four groups of rats were used to investigate the effect of tumor on the pharmacodynamics of phenobarbital hypnotic action: Group I—acute paraplegia, one day after the onset of paraplegia; Group II—sustained paraplegia, 4 days after the onset of paraplegia; Group III—systemic tumor mass, 21 days after inoculation of malignant cells in the hindlimb; and Group IV, controls—saline-treated rats.

The rats were infused intravenously with sodium phenobarbital at a constant rate of 48 mg/hr until they lost their righting reflex, while the rats were on a heating pad to maintain normal body temperature (7). At this time, CSF samples were obtained from the cisterna magna, and blood samples were collected from the abdominal aorta and frozen (−20°C) pending analysis. CSF specimens contaminated with blood were discarded.

To assess the effect of sustained paraplegia of 4 days' duration on the pharmacodynamics of PTZ-induced maximal seizures, the rats received an intravenous infusion of PTZ at a rate of 37 mg/hr until the onset of maximal convulsions, which were evidenced by tonic flexion on the forelimbs and, usually, tonic extension of the hindlimbs (9). Then, blood and brain samples were obtained and frozen pending analysis. In both investigations rectal temperature was monitored prior to drug infusion, and normal body temperature was maintained during drug infusion by placing the rats on a heating pad.

Analytical Procedures

Phenobarbital concentrations in serum and CSF were assayed by a high-performance liquid chromatography (HPLC) method previously described by Danhof and Levy (7), with certain modifications. The standard curve was linear in the range of 50–300 mg/L ($r > 0.998$).

The degree of protein binding (free fraction) of phenobarbital was determined by equilibrium dialysis of serum against an equal volume of sodium and potassium phosphate buffer (0.06 *M*, pH 7.4) containing 120 mg/L of phenobarbital, equilibrated for 6 hr at 37°C in Plexiglas cells, and separated by a cellulose membrane with a molecular exclusion limit of 12,000 to 14,000 Da (Vising cellulose tubing, Union Carbide, New York).

PTZ concentrations in serum and brain were determined by an HPLC method described by Ramzan and Levy (9) with certain modifications. Standard curve was linear in the range of 50–250 mg/L ($r > 0.998$).

Serum analysis of urea nitrogen levels, alanine aminotransferase activity, and total protein concentrations were performed using commercially available kits (Sigma Chemical Co., St. Louis, MO).

Statistical Analysis

Nonparametric tests were used in all cases. Differences among several groups were assessed by the Kruskal–Wallis test, and comparison between two groups' values was performed by the Mann–Whitney test. Statistical significance was determined at the $P < 0.05$ level.

RESULTS

Pharmacodynamics of Phenobarbital

The physiological and biochemical parameters of the rats that were used in this study are summarized in Table I. All the paraplegic rats appeared distinctly sick and had sig-

Table I. Characteristics of Tumor-Bearing Rats Used for the Study of the Pharmacodynamics of Phenobarbital Anesthesia[a]

Parameter	Control	Hindlimb tumor	Acute paraplegia	Sustained paraplegia
No. of rats	11	14	10	10
Weight (g)	159 ± 18	158 ± 14	168 ± 13	154 ± 13
Serum urea nitrogen (mg/dL)	18 ± 8	19 ± 8	24 ± 11	33 ± 12
Serum alanine aminotransferase (IU/L)	53 ± 18	40 ± 10	74 ± 52	59 ± 15
Serum total protein (g/dL)*	6.2 ± 0.5	4.4 ± 0.3**	4.8 ± 2.3**	5.0 ± 0.5**
Rectal temp. (°C)*	37.7 ± 0.3	37.5 ± 0.6	36.1 ± 0.5**	36.3 ± 0.6**

[a] Data are presented as mean ± SD.

* Significant difference between groups by Kruskal–Wallis test, $P < 0.01$.

** Significant difference from control group by Mann–Whitney test, $P < 0.05$.

nificantly lower body temperature. Total serum protein was significantly lower in all tumor-bearing rats. Serum urea nitrogen concentrations and serum alanine aminotransferase activity values were within the normal range in all the study groups. Three weeks after tumor inoculation into the right hindlimb, the mean diameter of the tumor mass measures was 3.5 ± 0.3 cm and the mean weight was 26.9 ± 8.5 g. There was a significant loss in actual body weight of all tumor-bearing rats, which was masked by the tumor weight.

Phenobarbital doses required to induce sleep, and drug concentrations in serum and CSF at the onset of the pharmacologic effect are presented in Table II. At loss of the righting reflex, the CSF phenobarbital concentration in animals with sustained paraplegia was significantly higher compared with that of control rats ($P < 0.05$). On the other hand, at the onset of hypnotic action no significant differences were found between CSF phenobarbital concentrations in the group with acute paraplegia and those in the control group. Similarly, no differences were noted between rats with hindlimb tumor and controls. The elevated CSF phenobarbital concentration required to induce sleep in the group of sustained paraplegia was not associated with an elevation in the total phenobarbital dose (normalized by weight) or with the total or free serum concentrations.

Pharmacodynamics of PTZ

The characteristics of rats with sustained paraplegia used for the study of the pharmacodynamics of PTZ-induced maximal seizures are summarized in Table III. Four days after the onset of paraplegia the rats appeared very sick. They were incontinent, had low body temperatures, and had significantly elevated serum urea nitrogen levels. The pronounced disease state did not affect the PTZ dose required to induce maximal seizures or the PTZ concentrations measured in the brain and serum of paraplegic rats at the onset of convulsions.

DISCUSSION

Pharmacodynamics of Phenobarbital

Little information is available on the effect of cancer on CNS sensitivity to the hypnotic action of anesthetic drugs, and even the few existing reports are contradictory.

In the present investigation the mere existence of the tumor had no effect on CNS sensitivity to the anesthetic action of phenobarbital. Thus, at the onset of loss of the righting reflex, phenobarbital CSF concentrations were similar in animals with hindlimb tumors, in rats with acute paraplegia, and in normal controls. Exceptional is the state of sustained paraplegia which was associated with diminished CNS sensitivity to the hypnotic action of phenobarbital as indicated by the higher CSF phenobarbital concentrations measured at the onset of hypnotic action. The reduced sensitivity may be related to changes in neuroactive mediators imposed in the brain by the sustained spinal cord compression. It was recently demonstrated that in neoplastic spinal cord compression, there is an increase in the turnover of serotonin along the spinal cord, evident also in distant noncompressed segments (4). It is possible that similar changes occur in the brain stem and that other neuroactive mediators are affected as well.

Since the pharmacodynamic effect was apparent only 4 days following the onset of paraplegia, a time-dependent mechanism, such as up- or down-regulation of receptors, may be involved, or accumulation of endogenous substances may reduce the receptor sensitivity to phenobarbital. Other pathophysiological mechanisms associated with the sustained paraplegia could contribute to the observed pharmacodynamic changes. They include intestinal and/or bladder dysfunction, hypoproteinemia, low body temperature, immobilization, and reduced food and water intake. Previous investigations evaluated the effect of similar pathophysiological states on the pharmacodynamics of phenobarbital-induced anesthesia. Hypoalbuminemia, secondary to nephrotic syndrome, and changes in drug binding to serum proteins had no effect on the pharmacodynamics of heptabarbital-induced anesthesia (11). Therefore, it is most likely that hypoalbuminemia did not account for the reduced sensitivity found in our study. Body temperature was similarly low 1 and 4 days after the onset of paraplegia and, therefore, could not contribute to the differences between the two paraplegic groups. On the other hand, impaired food and water intake secondary to intestinal hypomotility and immobilization may have played a part in the observed changes. Previous work by Zhi and Levy (12) showed that water deprivation for 24 or 48 hr did not alter the pharmacodynamics of the hypnotic action of phenobarbital. Therefore, reduced fluid

Table II. Effect of Neoplastic Tumor and Paraplegia Induced by Spinal Cord Compression on the Concentrations of Phenobarbital at the Onset of Anesthesia[a]

Parameter	Control	Hindlimb tumor	Acute paraplegia	Sustained paraplegia
Infusion time (min)	36.5 ± 7	34.5 ± 8.5	32.1 ± 7.1	32.5 ± 4.2
Dose (mg/kg)	180 ± 37	174 ± 38	154 ± 34	168 ± 20
Serum conc. (mg/L)				
Total	228 ± 37	200 ± 30	200 ± 33	246 ± 40
Free	140 ± 23	151 ± 23	136 ± 22	168 ± 27
CSF conc. (mg/L)[b,*]	125 ± 20 (6)	141 ± 25 (12)	140 ± 20 (5)	170 ± 31 (9)**

[a] Data are presented as mean ± SD.
[b] n as in Table I, except for CSF, with the value of n in parentheses.
* Significant difference between groups by Kruskal–Wallis test, $P < 0.01$.
** Significant difference from control group by Mann–Whitney test, $P < 0.05$.

Table III. Characteristics of Rats with Sustained Paraplegia and Its Effect on the Pharmacodynamics of PTZ-Induced Maximal Seizures

Parameter	Control	Sustained paraplegia
No. of rats	11	7
Weight (g)	294 ± 52	312 ± 33
Serum urea nitrogen (mg/dL)	10 ± 9	96 ± 58*
Rectal temp. (°C)	37.4 ± 0.6	34.2 ± 2.1*
Infusion time (min)	34.7 ± 6.2	33.5 ± 10.8
Dose (mg/kg)	73 ± 14	66 ± 22
Serum conc. (mg/L)	88 ± 17	99 ± 19
Brain conc. (mg/kg)	78 ± 9	85 ± 15

* Significant difference from control group by Mann–Whitney test, $P < 0.05$.

volume is probably not responsible for changes in brain sensitivity. Noteworthy is that food deprivation for 3 days was found to diminish CNS sensitivity to the hypnotic effect of phenobarbital (13). This is in agreement with the results of the present study. Although the rats' food was dispersed on the bottom of the cage and was readily available, starvation due to lack of appetite could affect the CNS sensitivity.

Phenobarbital CSF concentrations, measured at the onset of the pharmacologic end point, do not correlate with the total serum drug concentrations (see Table I). It should be noted that in all three tumor-bearing groups, the enlarging tumor induced hypoproteinemia. A low serum protein concentration resulted in reduced drug–protein binding and thereby in increased phenobarbital free fraction. Adjusting the serum drug concentration according to the respective free fraction minimized the differences found between drug concentrations in CSF and serum concentrations in the four study groups. However, it is important to note that phenobarbital CSF concentrations at the onset of the pharmacologic end point are clearly a better pharmacodynamic indicator than free serum concentrations, since the CSF is virtually protein-free, and CSF phenobarbital concentrations are in rapid equilibrium with the site of action, and, therefore, unaffected by pharmacokinetic variables (7).

Pharmacodynamics of PTZ

The effect of neoplastic spinal cord compression on the pharmacodynamics of PTZ-induced maximal convulsions was investigated, in an analogous experiment, to evaluate whether this disease state is proconvulsive. This is a very complex disease state that affects many biochemical and physiological variables that may shift the convulsive threshold in opposite directions. Our results indicate that paraplegia of 4 days' duration does not affect the concentration–effect relationship of PTZ-induced maximal seizures (Table III), but it remains unclear whether it represents the final balance of opposing fluctuations.

Elevated levels of serum urea nitrogen detected in the diseased animals may indicate either dehydration, impaired kidney function, or postrenal urinary tract obstruction. The effect of such disorders on the pharmacodynamics of PTZ was studied by Walker and Levy (15). They found that when high BUN values resulted from ureter ligation, there was a significant rise in CNS sensitivity to the convulsant action of PTZ. Otherwise, when caused by a nephrotoxic agent, a lower sensitivity to the convulsant substance was observed. They concluded that renal failure by itself diminish CNS sensitivity to the convulsant effect of PTZ, but it is counterbalanced by water retention and edema formation that produces the contrary effect that their tendency to induce convulsions (14). Apparently, in our experiments the bladder was filled with clotted blood but the outflow obstruction was incomplete since overflow incontinence was evidenced with constant urinary draining. Unlike the effect of food withdrawal on the pharmacodynamics of phenobarbital, starvation is not expected to affect the pharmacodynamics of PTZ-induced seizures (13). On the other hand, reduced body temperature observed in our rats is another potential variable that may affect PTZ-induced convulsion threshold (15). The fact that no changes were detected in the concentration–convulsant effect relationship albeit severe pathophysiological changes indicates that it is most likely related to the counterbalancing effects of various events, rather than to an unaffected CNS sensitivity.

It should be noted that although in both experiments sustained paraplegia was similarly defined (i.e., 4 days after onset of paraplegia), the physiological status differed markedly. Pronounced reduction in body temperature and marked elevation in serum urea nitrogen concentrations were noted in the PTZ investigation, unlike the findings in the phenobarbital investigation (Tables I and III). The interval between tumor inoculation and onset of paraplegia and the pace of neurological deterioration were longer in the phenobarbital study than in the PTZ investigation. In addition, the bladders were found to be full of coagulated blood in the PTZ study but not in the phenobarbital study. These differences may be related either to tumor heterogeneity expressed as variations in tumor growth or to the differential effects of sex hormones on tumor development since the animal's sex differed in the two experiments.

The relevance of these animal studies to humans may be questionable, however, unlike the marked differences in drug doses and pharmacokinetic parameters usually observed between rats and human, drug concentrations at the onset of a similar pharmacologic effect are often similar (16). It is therefore suggested that the sensitivity of the CNS to depressant and stimulant drugs is not likely to be affected by spinal neoplastic cord compression, yet sustained paraplegia with its associated pathophysiological changes may reduce CNS sensitivity to the hypnotic action of barbiturate.

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