Biochemical Pharmacology, Vol. 20, pp. 3247–3254. Pergamon Press, 1971. Printed in Great Britain

ENHANCEMENT OF PENTOBARBITAL NARCOSIS BY DRUGS COMPETING ON THE SERUM PROTEIN BINDING

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(*Received* 12 *March* 1971; *accepted* 27 *April* 1971)

Abstract—Prior subcutaneous administration of sulfaethylthiazole (SET), sulfamethazine (SMZ), sulfanilamide (SNA), salicylic acid (SA), doxycycline (DOXI), *p*-aminosalicylic acid (PAS) is able to enhance the intraperitoneal pentobarbital (PB) narcosis in mice.

Such narcosis enhancement seems to be connected with competition between these drugs and PB for serum proteins, which results in an increase of the unbound PB and consequently a higher PB cerebral concentration. This higher concentration may be attributed to competition for serum protein binding, since the PB metabolism is unaffected by SET, SMZ, SNA, SA, DOXI and PAS.

THE DISTRIBUTION of a drug can be influenced by changes in the serum level of its unbound fraction.[1–2] Changes in the serum level of a drug can be obtained by concomitant administration of another drug competing with the first one in serum protein binding.

Thus when triacetyloleandomycin with sulfamethazine,[3] or sulfaethylthiazole with 6-methylene-5-oxytetracycline, pentobarbital, or sodium salicylate,[4] or sulfadimethoxine with 6-methylene-5-oxy-tetracycline,[4] are administered, the levels of the unbound serum fractions of each drug, as well as their extrahematic concentrations are generally higher than those obtained when the two drugs are administered separately. It is also known that pentobarbital competes with 6-methylene-5-oxytetracycline, sulfaethylthiazole and cyanocobalamin.[5]

Furthermore, it is of interest to determine whether pentobarbital narcosis can be strengthened by drugs competing with pentobarbital on the serum protein binding.

MATERIALS AND METHODS

The following compounds were used: pentobarbital (PB), sulfaethylthiazole (SET), sulfamethazine (SMZ), sulfanilamide (SNA), salicylic acid (SA), doxycycline (DOXI), *p*-aminosalicylic acid (PAS), bovine serum albumin (fraction V-Sigma Chem. Co.) purified: (1) from fatty acids by extracting the protein with 5% glacial acetic acid in isooctane, according to Goodman;[6] (2) from metal ions by adding ethylenediamine tetracetic acid (EDTA) and then dialyzing it until all traces of EDTA had disappeared. Male albino mice (*Mus musculus*) and male mongrel dogs were used.

A.1. *Binding capability of serum albumin to PB, SET, SMZ, SNA, SA, DOXI, PAS*

The serum albumin, PB, SET, SMZ, SNA, SA, DOXI and PAS were dissolved in 0·1 M phosphate buffer, pH 7·2.

The concentration of the protein was $5{\cdot}7 \times 10^{-4}$ M, that of PB, SET, SMZ, SNA, DOXI and PAS was $1{\cdot}2 \times 10^{-4}$ M. The equilibrium dialysis technique described by Klotz, Walker and Pivan[7] was used. The dialysis was carried out at +4° for 24 hr, after which the outside portion of dialysis system was assayed for the presence of SET, SMZ, SNA, PAS by the method of Bratton and Marshall,[8] and of DOXI, PB, SA by a spectrophotometric method. The binding capability of PB, SET, SMZ, SNA, SA, DOXI, PAS was calculated on the basis of the number of moles bound per mole of protein (r).

2. *Competition between pentobarbital and SET, SMZ, SNA, SA, DOXI, PAS*

(a) Two ml of serum albumin ($5{\cdot}74 \times 10^{-4}$ M) in 0·1 M phosphate buffer (pH 7·2) combined or not with SET, SMZ, SNA, SA, DOXI, PAS at concentrations ranging from $7{\cdot}0 \times 10^{-4}$ M to $3{\cdot}5 \times 10^{-3}$ M were dialyzed against 6 ml of a solution of PB ($1{\cdot}20 \times 10^{-4}$ M).

(b) Two ml of serum albumin ($5{\cdot}74 \times 10^{-4}$ M) in 0·1 M phosphate buffer (pH 7·2) combined or not with PB at concentrations ranging from $0{\cdot}40 \times 10^{-4}$ M to $1{\cdot}0 \times 10^{-3}$ M were dialyzed for 24 hr at +4° against 6 ml of a solution of SET, SMZ, SNA, SA, DOXI, PAS ($1{\cdot}76 \times 10^{-4}$ M) after which the outside portion of the dialysis system was assayed for the presence of PB or SET, SMZ, SNA, PAS, DOXI, SA. The binding capability was calculated as described above.

3. *Binding capability of SET, SA, DOXI, PAS to serum of pentobarbital treated dogs*

Male dogs weighing $14 \pm 2{\cdot}3$ kg were i.v. narcotized with PB (30 mg/kg); 60 min before and 60, 120, 300 min after the pentobarbital treatment, samples of blood were taken from each dog, and the serum binding capability to $1{\cdot}76 \times 10^{-4}$ M SET, SA, DOXI, PAS was evaluated. PB serum levels were evaluated by the Goldbaum method;[17] the percentage of total proteins and of the various serum protein fractions was also evaluated.

B. *Modifications on PB narcosis caused by SET, SMZ, SNA, SA, DOXI, PAS*

Male mice weighing 21–23 g were divided into seven groups, each group consisting of 20 animals. One group, the controls, was injected s.c. with 10 ml/kg of NaCl 0·9% while the other groups received s.c. respectively $1{\cdot}4 \times 10^{-4}$ moles/kg of SET, SMZ, SNA, SA, DOXI, PAS. After 30 min all the animals were injected with 40 mg/kg of PB i.p.; the narcosis onset and the sleeping times were recorded.

C. *Modifications in PB metabolizing capability of liver homogenate caused by SET, DOXI, SA*

Liver was obtained from decapitated mice and immediately homogenized in ice-cold 0·25 M sucrose.

The ability of liver homogenate to metabolize PB was assayed with the technique described by Kuntzman *et al.*[9] in the presence of a cofactor mixture consisting of 0·5 ml of glucose-6-phosphate dehydrogenase (5 Kornberg units), 0·2 ml of 0·03 M glucose-6-phosphate, 0·1 ml of NADP solution (4 mg/ml), 0·1 ml of NAD solution (4 mg/ml), 0·2 ml of 0·01 M ATP, 0·2 ml of 0·6 M nicotinamide, 0·1 ml of 2 M KCl, 0·1 ml of 0·1 M $MgCl_2$ and 1·0 ml of 0·1 M KH_2PO_4–K_2HPO_4 buffer at pH 7·4.

The PB was determined by spectrophotometric method.[17]

D. *Modifications on PB brain level in mice caused by SET*

Male mice weighing 21–23 g were divided into two groups of 12 animals each. One group, the controls, was injected s.c. with 10 ml/kg of NaCl 0·9% solution, while the other group received SET solution at $1{\cdot}4 \times 10^{-4}$ moles/kg.

After 30 min, each of the animals in the two groups was injected with PB (40 mg/kg i.p.). The PB concentration in the brain was determined 27 min after the barbiturate treatment.

The animals were decapitated and arranged in groups of three mice each. Equal samples of brain from each of the three animals were pooled. The PB concentration in the brain was determined by spectrophotometric method.[17]

RESULTS

SA is the drug with the most extensive binding capability to serum albumin, followed by those of DOXI, SET, PAS, PB, SMZ, and SNA (Fig. 1). As shown in Fig. 2, SET, SMZ, SNA, SA, DOXI, PAS when added simultaneously with PB to serum

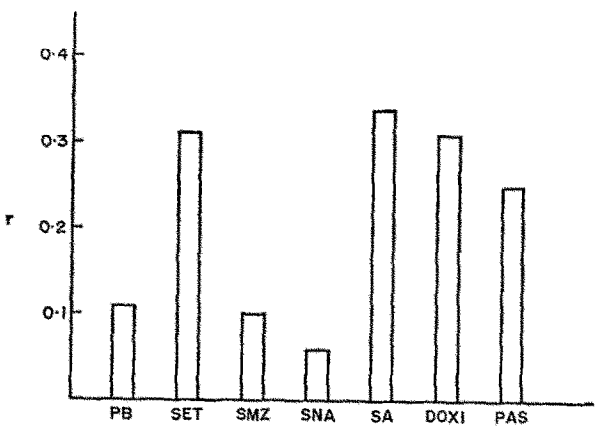

FIG. 1. Binding capability of PB, SET, SMZ, SNA, SA, DOXI, PAS (at $1{\cdot}20 \times 10^{-4}$ M concn) to bovine serum albumin ($5{\cdot}74 \times 10^{-4}$ M). Ordinate = r: moles of PB, or SET, or SMZ, or SNA, or SA, or DOXI, or PAS bound per mole of protein.

PB	= pentobarbital.	SA	= salicylic acid.
SET	= sulfaethylthiazole.	DOXI	= doxycycline.
SMZ	= sulfamethazine.	PAS	= *p*-aminosalicylic acid.
SNA	= sulfanilamide.		

albumin compete with the latter in the interaction. The degree of competition depends upon the drug concentration ratio. It was also observed that greater reduction of the PB binding is shown by SET, followed by PAS, SMZ, SA, SNA and DOXI. On the other hand, PB is able to reduce mainly the binding capability of SNA and in decreased order of SMZ, SET, DOXI, PAS and SA.

The binding capability of PAS, SET, DOXI, SA, to dog serum obtained 1–2–5 hr after PB treatment is reduced, compared to the binding capability observed before PB treatment. The reduction seems to be in connection with the PB serum concentration (Fig. 3).

TABLE 1. EFFECT OF SET, SMZ, SNA, SA, DOXI, PAS ($1{\cdot}37 \times 10^{-4}$ moles/kg s.c.) ON THE NARCOSIS ONSET AND SLEEPING TIME OF MICE GIVEN PENTOBARBITAL (40 mg/kg i.p.)

Group *n*	Pretreatment	Moles $\times 10^{-4}$	No. of mice	Narcosis onset (sec ± S.E.)	P vs. the group 1	Sleeping time (sec ± S.E.)	P vs. the group 1
1	None	—	20	376 ± 32	—	2625 ± 390	—
2	SET	1·37	20	313 ± 20	<0·05	4641 ± 427	<0·005
3	SMZ	1·37	20	335 ± 24	<0·20	4721 ± 371	<0·005
4	SNA	1·37	20	296 ± 15	<0·0125	4467 ± 303	<0·005
5	SA	1·37	20	309 ± 19	<0·05	4265 ± 452	<0·005
6	DOXI	1·37	20	300 ± 21	<0·025	5938 ± 607	<0·005
7	PAS	1·37	20	305 ± 22	<0·05	4459 ± 303	<0·005

SET = sulfaethylthiazole.
SMZ = sulfamethazine.
SNA = sulfanilamide.
SA = salicylic acid.
DOXI = doxycycline.
PAS = *p*-aminosalicylic acid.

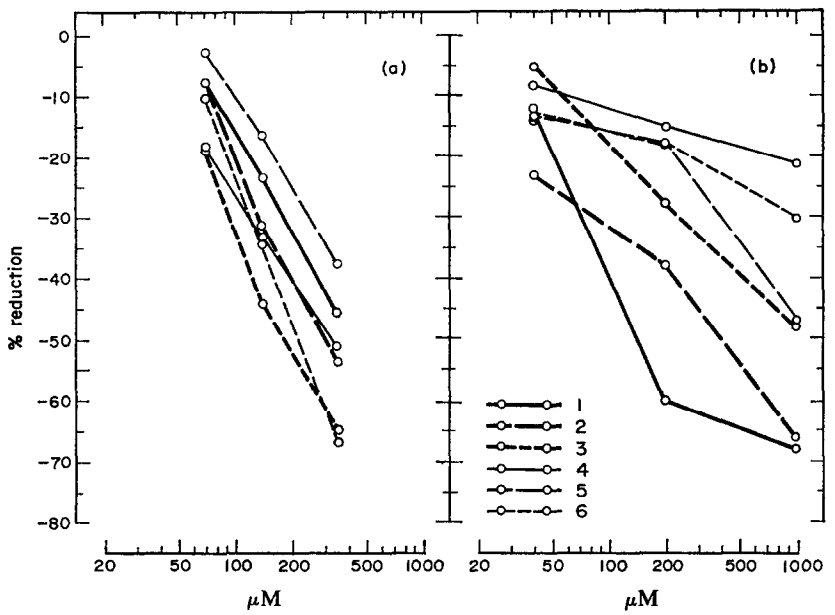

FIG. 2. (a) Percentage reduction in the bovine serum albumin ($5{\cdot}7 \times 10^{-4}$ M) binding with PB ($1{\cdot}20 \times 10^{-4}$ M) in the presence of different concentrations of SNA (1), SMZ (2), SET (3), SA (4), DOXI (5), PAS (6).

(b) Percentage reduction in the bovine serum albumin ($5{\cdot}7 \times 10^{-4}$ M) binding with SNA (1), SMZ (2), SET (3), SA (4), DOXI (5), PAS (6) (at $1{\cdot}76 \times 10^{-4}$ M concn) in the presence of different concentrations of PB.

PB = pentobarbital.
SNA = sulfanilamide.
SMZ = sulfamethazine.
SET = sulfaethylthiazole.
SA = salicylic acid.
DOXI = doxycycline.
PAS = *p*-aminosalicylic acid.

TABLE 2. EFFECT OF SET, SA, DOXI ON THE PB METABOLIZING CAPABILITY OF LIVER HOMOGENATE

Drug	M/l. $\times 10^3$	PB added γ	Metabolized PB γ/100 mg/60 min $\pm$ S.E.
—	—	250	18·3 ± 2·3
SET	1·76	250	18·9 ± 2·1
SET	3·53	250	19·8 ± 2·4
SA	3·53	250	17·5 ± 2·2
DOXI	3·53	250	20·3 ± 2·5

SET = sulfaethylthiazole.
SA = salicylic acid.
DOXI = doxycycline.
PB = pentobarbital.

SET, SMZ, SNA, SA, DOXI and PAS s.c. injected to mice 30 min before the i.p. treatment of PB, reduce the narcosis onset and increase the sleeping time (Table 1, Fig. 4).

SET, SA and DOXI do not affect PB metabolism by liver homogenate (Table 2).

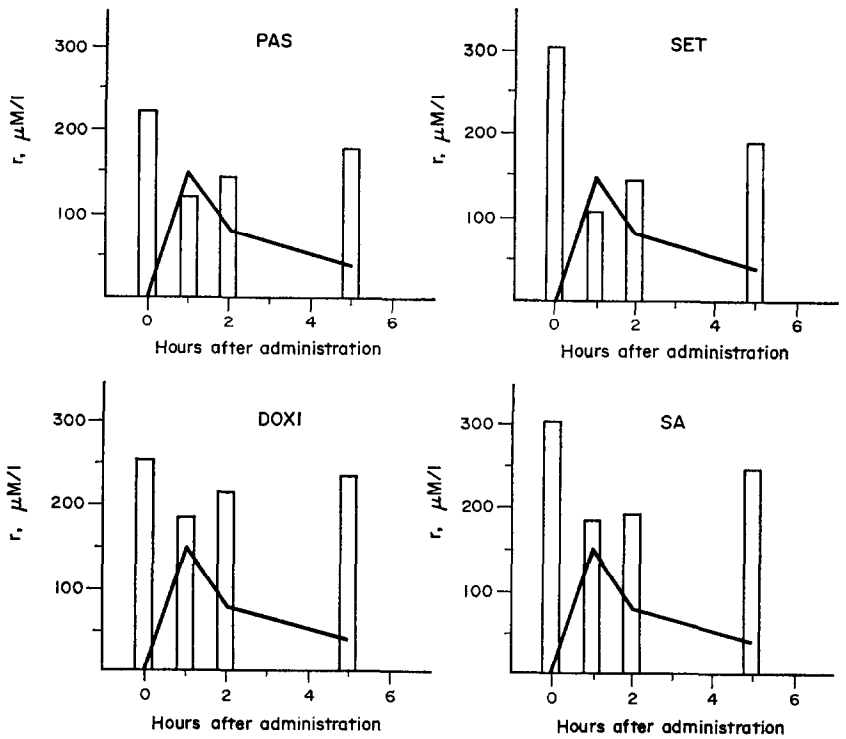

FIG. 3. Serum levels (solid line) of PB (μM/l.) in dogs after i.v. administration of 30 mg/kg of the drug and (vertical bar) binding capability (μmoles bound per gram of total proteins = *r*) of PAS, SET, DOXI, SA ($1{\cdot}76 \times 10^{-4}$ M) with dog serum. The sera were collected before and 1–2–5 hr after i.v. administration of PB (30 mg/kg).

PAS = *p*-aminosalicylic acid.
SET = sulfaethylthiazole.
DOXI = doxycycline.
SA = salicylic acid.
PB = pentobarbital.

TABLE 3. EFFECT OF SET (40 mg/kg s.c.) ON THE PB BRAIN LEVELS IN MICE GIVEN PB (40 mg/kg i.p.) 27 min BEFORE

Pretreatment	PB brain level $\gamma \pm$ S.E.	P
None	$19{\cdot}9 \pm 1{\cdot}3$	—
SET	$35{\cdot}5 \pm 2{\cdot}5$	$<0{\cdot}001$

SET = sulfaethylthiazole.
PB = pentobarbital.

PB brain levels (Table 3) in mice pretreated with SET are higher ($35{\cdot}5 \pm 2{\cdot}5$ γ/g) than those of mice which have not been pretreated ($19{\cdot}9 \pm 1{\cdot}3$ γ/g).

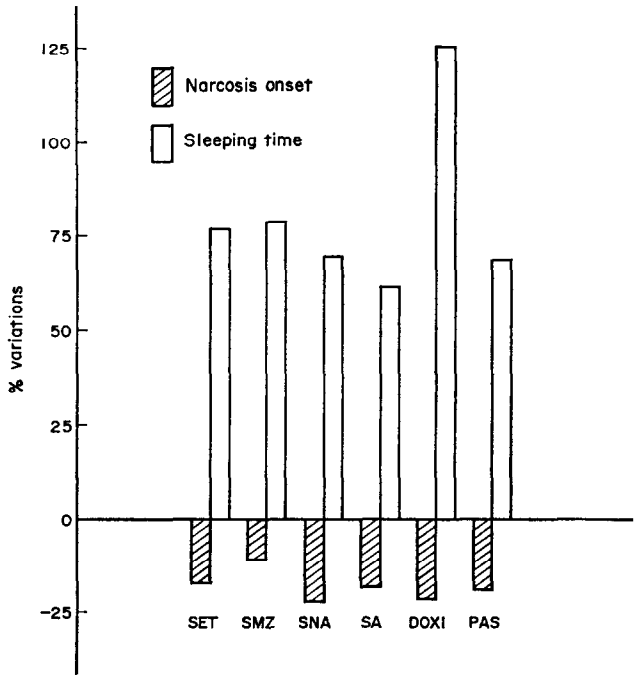

FIG. 4. Per cent variations in narcosis onset and sleeping time of mice given SET, SMZ, SNA. SA, DOXI, PAS ($1{\cdot}37 \times 10^{-4}$ moles/kg s.c.) 30 min before PB (40 mg/kg i.p.).

SET	= sulfaethylthiazole.	DOXI	= doxycycline.
SMZ	= sulfamethazine.	PAS	= *p*-aminosalicylic acid.
SNA	= sulfanilamide.	PB	= pentobarbital.
SA	= salicylic acid.		

DISCUSSION

PB narcosis is significantly enhanced by SET, SMZ, SA, SNA, DOXI, PAS s.c. administered 30 min before the i.p. injection of the barbiturate.

The enhancement seems related to a higher PB cerebral concentration, as we observed when we injected SET before PB.

Such higher PB cerebral concentration seems not to be connected with an inhibition of the PB metabolism related to the liver, since SET, SA and DOXI do not influence the PB metabolization by mice liver homogenates.

On the other hand we observed a competition between PB and SET, SMZ, SNA, SA, DOXI, PAS on the interaction with serum albumin; it is therefore possible to suggest that the PB narcosis enhancement could be related to the competition on the serum protein binding and consequently to the higher serum unbound level of PB. This hypothesis is supported also by the decrease in the narcosis latency time when PB is injected after SET, SMZ, SNA, SA, DOXI, PAS.

Probably the higher unbound PB can more easily diffuse to the central nervous system, where it is retained because of its lipophilia.

Many researchers observed a PB narcosis enhancement by different drugs; however, such enhancement is generally related to some synergism on the depressive action on the central nervous system[16] or to some inhibition of the PB metabolism.[10–16]

Our results demonstrate that the PB narcosis enhancement can also be related to some competition on the serum protein binding by drugs which do not have any depressing action on the central nervous system or any action affecting the PB metabolism.

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