Depression of the MSR and increase in the DRP by pentobarbital

Dose of pentobarbital (mg/kg)	Semicarbazide-treated cats MSR[a]	DRP	Saline-treated cats MSR[a]	DRP
10	22 ± 7 (6)	No effect (3) or mild increase in amplitude (3)	31 ± 6 (8)	Increase in amplitude and/or duration (8)
20	10 ± 8 (6)	No effect (6)	12 ± 7 (4)	Increase in duration (4)

[a] The depressed MSR is expressed as mean % of control followed by the standard error and number of experiments in parentheses. The size of the MSR $4^1/_2$ h after semicarbazide or saline is taken as control.

After cannulating the trachea and ligating carotid arteries of cats initially anesthetized with ether, the spinal cord was severed at the level of the atlanto-occipital junction. Ether was then discontinued and the cats immediately placed under artificial respiration, with end tidal CO_2 levels controled at 3.5–4%. The brain was made ischemic by local pressure applied on the vertebral arteries for 5 min. The lumbosacral cord was exposed, the dura sectioned, and ventral L_6, L_7, and S_1, and all contralateral dorsal and ventral roots were cut. The exposed tissues were covered with warm mineral oil, the temperature of which was maintained at 37 °C thermostatically. The ipsilateral hamstring nerves were dissected out, crushed distally, and placed on platinum hook electrodes for stimulation (12 c/min) in a pool of warm mineral oil. All other leg nerves were sectioned. Reflex transmission was recorded in ipsilateral ventral L_7, while the DRP was recorded from a dorsal L_6 rootlet, with the time constant of the preamplifier set at 1 sec.

The effects of i.v. administration of semicarbazide (200 mg/kg) on spinal cord potentials have already been described[6,7]. The most pronounced effect was the gradual depression of the DRP (specifically DRV), which was reduced in area to below 20% of control size and sometimes completely blocked within $4^1/_2$ h of drug administration. The ventral root discharge was usually facilitated, as shown by the increase in size of the monosynaptic (MSR) and polysynaptic (PSR) responses in 4 out of 6 cats. Pentobarbital was administered i.v. 270 min after semicarbazide, at a time when GABA has been shown to be completely depleted from the spinal cord[6]. In all 6 experiments, it resulted in immediate depression of the MSR and PSR. A dose of 10 mg/kg depressed the MSR to 22 ± 7% of control size, while 20 mg/kg depressed it to 10 ± 8% (Table). In 3 of these experiments, pentobarbital (10 mg/kg) failed to increase the DRP, while in the remaining 3 experiments, it mildly increased its amplitude. With larger doses (20 mg/kg or more), it uniformly failed to prolong its duration. All these effects were obtained irrespective of the strength of stimulation of the hamstring nerve (3- and 12-times threshold).

In control experiments where the cats were given saline in place of semicarbazide, the administration of pentobarbital (10-20 mg/kg) $4^1/_2$ h later resulted in depression of the MSR and PSR (Table), a well-defined increase in the amplitude of the DRP after smaller doses, and a definite prolongation of its duration after larger doses (20 mg/kg).

Thus it can be seen that pentobarbital is at least equally depressant in the presence or absence of GABA. Its depressant action on ventral root discharge, therefore, is not mediated, even partly, by this inhibitory transmitter candidate. It probably results from a direct effect on synaptic elements, decreasing the release of the primary afferent transmitter in smaller doses[8,9], and reducing the excitability of motoneuronal membranes in larger doses[10,11].

Another interesting conclusion is that the depressant action of pentobarbital on ventral root discharges can be dissociated from its ability to increase primary afferent depolarization, since in some experiments, depression of reflex transmission in the ventral root occurred in the absence of a concomitant increase in the size of the DRP[12].

Résumé. Le pentobarbital fait diminuer la transmission à travers le relais monosynaptique de la moelle épinière chez les chats décapités dépourvus de GABA sous un traitement au semicarbazide, et ceci par un degré équivalent à celui des expériences de contrôle.

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Phenobarbital Specific Antisera and Radioimmunoassay

Antibodies developed against drug haptens have been used in immunoassays of drugs in biological fluids[1,2]. We now report the procedures for developing an antibody which is highly specific to phenobarbital (5-ethyl-5-phenylbarbituric acid) and can be used in radioimmunoassay to detect picomole levels of this drug in biological fluids. The significant difference in the specificity of this antibody and that of antibody produced by SPECTOR and FLYNN[2] is of particular interest.

The barbiturate-protein conjugate was made by dissolving 1.25 g of 5-phenyl-5-(4-aminobutyl) barbituric acid hydro-chloride containing a small amount of

5-phenyl-t-(4-aminobutyl) barbituric acid-C^{14} tracer into 20 ml water containing 0.68 g of bovin serum albumin (BSA). The pH of the mixture was adjusted to 6.5 and 3.83 g of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in 6 ml water was then added with stirring. The reaction was terminated after 3.5 h by dialyzation against distilled water. The degree of conjugation was calculated from the amounts of the tracer coupled to the protein to be 33 molecules barbiturates per BSA macromolecule (molecular weight 68,000). (Figure 1).

The rabbits immunized with the conjugate in 50% complete Freund's adjuvant in saline were bled at the 7th week. The titers of the antisera were determined by the incubation of serial dilutions of antisera (0.1 ml each) with 0.11 picomole (1400 cpm) of phenobarbital-H^3 at 4°C overnight and then precipitated with a saturated ammonium sulfate solution for the separation of the free from the bound. The free labels were counted in a Nuclear Chicago spectrometer.

Antisera of a 1:750 dilution were used for radioimmunoassay of phenobarbital and cross reactivities studies. A 0.01 *M* phosphate buffer (pH 7.4) in saline containing 0.5% BSA and 0.05% human γ-globulin was used as a diluent. For the assays of urine or plasma samples, 0.1 ml of samples with various amounts of phenobarbital was incubated with 0.1 ml of antiserum and 1400 cpm of phenobarbital-H^3. Typical results are shown in Figure 2.

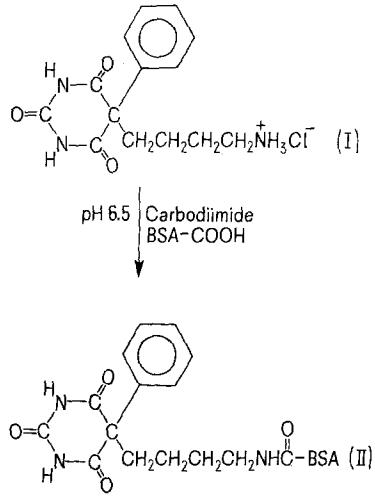

Fig. 1. Conjugation of 5-phenyl-5-(4-aminobutyl) barbituric acid hydrochloride to bovin serum albumin (BSA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide is used as a coupling agent.

The high sensitivity of our assay is related to the high affinity of the antibody obtained. It has been suggested[3] that a conjugate of high hapten to protein ratio is necessary for the production of antibodies of good affinity. A comparison of our hapten-protein ratio with that of SPECTOR and FLYNN[2] seems to indicate the importance of this factor.

The antibody has no significant cross reaction with amobarbital, butabarbital, pentobarbital, and hexobarbital; except secobarbital which shows a cross reactivity[4] of 0.005. These barbiturates differ from phenobarbital only by the substituents on the C-5 position, except hexobarbital which has an additional substituent on the N-1 position. The ability of the antibody to differentiate phenobarbital from the other barbiturates indicates the importance of the configuration of the hapten on the conjugate[5,6]. The high specificity of the antibody can be attributable to the fact that the hapten molecules are bridged to the protein at the C-5 position, with the bridge chain approximately bisecting the hapten molecule into 2 moities – the barbituric acid ring on one side and the phenyl ring on the other side. Thus the antibody produced from this conjugate recognizes not only the barbituric acid ring but also the phenyl ring as well. The relatively long bridge chain in this conjugate probably is the other factor which enhances the specificity of the antibody.

Résumé. On a développé un anticorps d'une grande affinité et spécifité envers le phénobarbital en inoculant des lapins avec une solution de barbiturateprotéide, synthétisée d'acide barbiturique de 5-phényl-5-(4-aminobutyl) et d'albumine de sérum bovin par du carbodiimide. Usant de cet anticorps comme radioimmunoeassai, on peut mesurer jusqu'à 1 picomole de phénobarbital par ml de fluides biologique sans que d'autres barbiturates y opposent une réaction significative.

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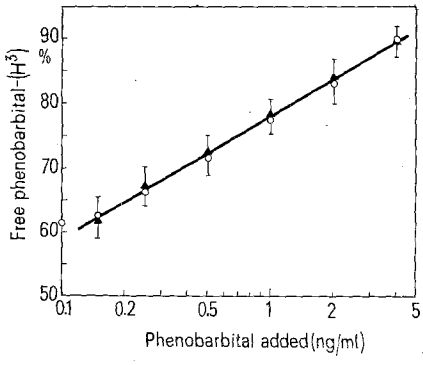

Fig. 2. Displacement of the phenobarbital-H^3 by various amounts of unlabeled phenobarbital in urine (△) and plasma (○) samples. The vertical bars at each test point indicate the standard deviations of the percentage free phenobarbital-H^3. Each point in the figure represents an average of at least 8 runs.

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[4] Cross reactivity = (The amounts of phenobarbital needed to displace 50% of phenobarbital H^3 from binding to the antibody)/(The amounts of barbiturate needed to displace 50% of phenobarbital-H^3 from binding to the antibody).

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