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## Effects of Trimethadione, Diphenylhydantoin and Chlordiazepoxide on After-Discharges in Brain of Cat. (28176)

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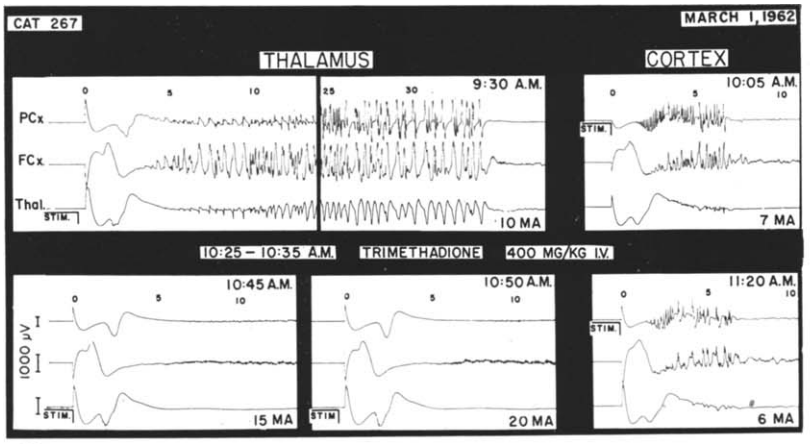
Hunter and Jasper(5) found that stimulation of intralaminar nuclei in the thalamus of the cat produced behavioral arrest, accompanied by a 3 per second spike and slow wave pattern in the EEG. Both behavioral and electrographic effects resembled those seen in petit mal epilepsy in man.

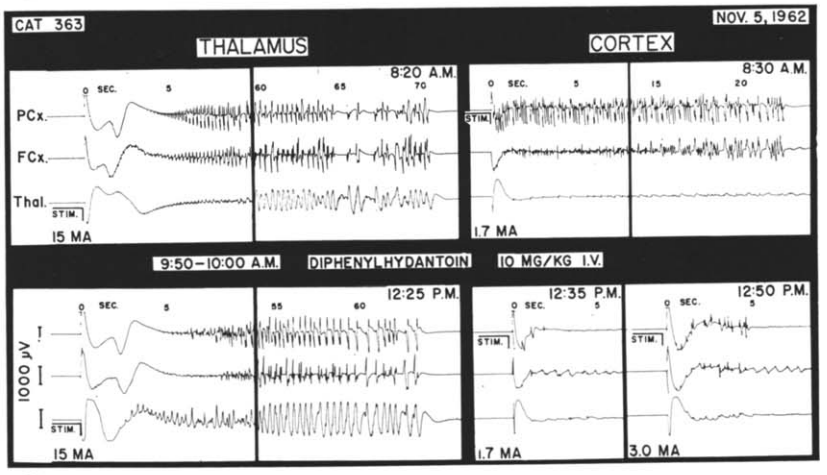
Trimethadione is clinically effective in petit mal epilepsy(13). If this type of seizure originates in the intralaminar nuclei, trimethadione should depress this part of the brain. We have tested the effects of trimethadione on the intralaminar central lateral nucleus of the cat, as well as on the adjacent medial dorsal nucleus, cortex and hippocampus. The effects of trimethadione were compared with those of diphenylhydantoin and chlordiazepoxide (Librium®).

*Methods.* Forty-one cats were prepared under ether anesthesia. The trachea was cannulated for artificial respiration and the left femoral vein for drug injection. Bipolar concentric electrodes were inserted with the aid of a stereotaxic instrument in central lateral or medial dorsal nuclei of thalamus, on frontal and parietal cortex (ectosylvian gyrus), and in anterior and posterior hippocampus. All wound edges were infiltrated with procaine. The ether was withdrawn and the cat immobilized with decamethonium ($C_{10}$).

Stimulation was with a Grass S-4 Stimulator. Parameters for medial dorsal nucleus, cortex and hippocampus were pulse duration 2 msec, frequency 40 per sec, duration of stimulation 5 sec, interval between stimulations 5 min. Initial intensity was 1 volt; this was increased by increments of 1 or 2 volts until an after-discharge appeared in the EEG, recorded on a Grass III-C electroencephalograph. Current was monitored with a Tektronix 502 oscilloscope. Current at threshold voltage was recorded as "threshold cur-


CAT 267
MARCH 1, 1962
THALAMUS
CORTEX
9:30 A.M.
10:05 A.M.
PCx.
FCx.
Thal.
STIM.
10 MA
7 MA
10:25 – 10:35 A.M.
TRIMETHADIONE
400 MG/KG I.V.
10:45 A.M.
10:50 A.M.
11:20 A.M.
1000 μV
15 MA
20 MA
6 MA

1


CAT 363
NOV. 5, 1962
THALAMUS
CORTEX
8:20 A.M.
8:30 A.M.
PCx.
FCx.
Thal.
STIM.
15 MA
1.7 MA
9:50 – 10:00 A.M.
DIPHENYL-HYDANTOIN
10 MG/KG I.V.
12:25 P.M.
12:35 P.M.
12:50 P.M.
1000 μV
15 MA
1.7 MA
3.0 MA

2

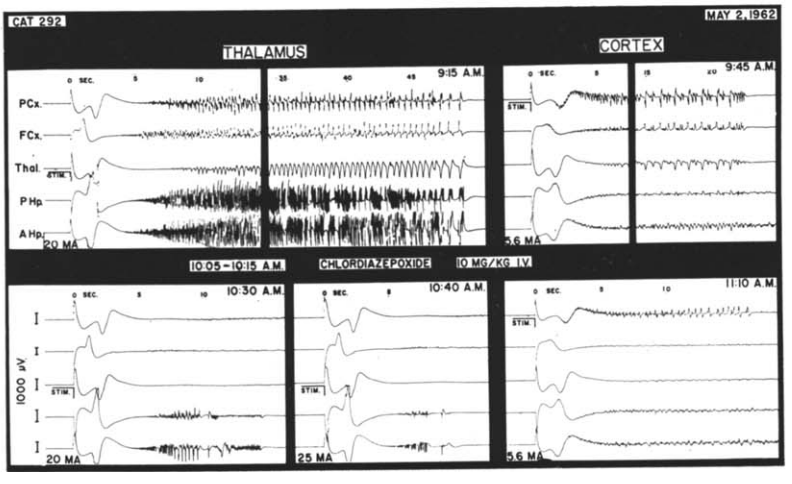

CAT 292
MAY 2, 1962
THALAMUS
CORTEX
9:15 A.M.
9:45 A.M.
PCx.
FCx.
Thal.
P Hp.
A Hp.
20 MA
5.6 MA
10:05 – 10:15 A.M.
CHLORDIAZEPOXIDE
10 MG/KG I.V.
10:30 A.M.
10:40 A.M.
11:10 A.M.
1000 μV
20 MA
25 MA
5.6 MA

FIG. 1. Trimethadione, 400 mg/kg i.v., depresses thalamus but not cortex. Before drug, stimulation of central lateral nucleus at 10 ma induces 35 sec after-discharge (upper left); after drug, no response in thalamus at 15 ma or 20 ma (lower left). Before drug, stimulation of parietal cortex at 7 ma induces 7 sec after-discharge (upper right); after drug 6 ma stimulation induces 7 sec after-discharge (lower right). EEG leads are parietal cortex, frontal cortex, thalamus. "Stim." marks final part of stimulation period and shows area stimulated; pens turned off to prevent their being damaged during stimulation. Numbers at top indicate time in sec since end of stimulation. Paper speed 1 cm per sec.

FIG. 2. Diphenylhydantoin, 10 mg/kg i.v., depresses cortex but not thalamus. Stimulation of central lateral nucleus at 15 ma induces after-discharge of 70 sec before drug (upper left) and 63 sec after drug (lower left). Stimulation of parietal cortex at 1.7 ma before drug induces after-discharge of 23 sec (upper right); after drug, after-discharge of 2 sec at 1.7 ma and 5 sec at 3.0 ma (lower right). Arrangement as in Fig. 1.

FIG. 3. Chlordiazepoxide, 10 mg/kg i.v., causes marked depression of thalamus. Before drug, stimulation of central lateral nucleus at 20 ma induces 49 sec after-discharge (upper left); after drug, no response in thalamus at 20 ma or 25 ma (lower left). Stimulation of parietal cortex at 5.6 ma induces after-discharge of 23 sec before drug (upper right), 17 sec after drug (lower right). Lower leads are posterior and anterior hippocampus; other arrangements as in Fig. 1.

rent." The voltage was now doubled to produce a supra-threshold response; amplitude and duration of after-discharge were measured on the EEG record.

The central lateral nucleus was stimulated with 10 msec pulses, frequency 5 per sec, for 15 sec. Stimulation began at 5 volts and was increased in steps of 5 volts until an after-discharge appeared. To avoid damaging tissue with high current flow, duration and amplitude measurements for this area were made at threshold rather than at twice threshold.

Drugs were dissolved in distilled water; control tests were made with saline. To minimize cardiovascular effects, all solutions were diluted to 10 ml and infused over a 10 minute period. Two complete sets of measurements for each brain area were made before injection, and 2 sets after injection. Mean pre- and post-injection values were determined for each parameter. Two sets of statistical analyses were made by the "t test": 1) in the control series, pre-injection values were compared with post-injection values; 2) the change following injection of each drug was compared with the change following injection of saline. Electrode locations were confirmed histologically by the Klüver-Barrera technique.

*Results* (Table I, Fig. 1-3). *Saline controls:* There was no significant difference between pre- and post-injection values in 8 of the 9 parameters measured. There was a just significant *fall* in threshold of central lateral nucleus of the thalamus ($p = 0.05$-$0.02$); in contrast, some of the drugs produced highly significant *rises* in this threshold ($p < 0.01$).

*Trimethadione,* 100 mg/kg, significantly in-

TABLE I. Statistically Significant Drug Effects.*

| Site of stimulation | Drug | Dose, mg/kg i.v. | Change in response† |
|---|---|---|---|
| | | | Threshold current, milliamp |
| Thalamus | Saline | — | − 3.0 ± .5 |
| | Trim. | 100 | + 3.1 ± 1.2 |
| | " | 400 | +12.5 ± 2.1 |
| | Diph. | 20 | + 3.7 ± 2.3 |
| | Chlo. | 10 | + 8.0 ± .5 |
| | " | 40 | +10.6 ± 2.1 |
| Cortex | Saline | — | − .33± .14 |
| | Diph. | 20 | + 5.8 ± 1.0 |
| | | | Duration of after-discharge, sec |
| Thalamus | Saline | — | +10.2 ± 5.8 |
| | Trim. | 400 | −29.8 ± 4.6 |
| | Diph. | 20 | −33.7 ± 4.9 |
| | Chlo. | 10 | −26.2 ± 3.0 |
| | " | 40 | −42.2 ± 4.6 |
| Cortex | Saline | — | + 1.4 ± 2.8 |
| | Diph. | 10 | −11.8 ± 1.1 |
| | " | 20 | −13.2 ± 3.4 |
| Hippocampus | Saline | — | + 6.3 ± 2.2 |
| | Diph. | 10 | −11.2 ± 3.9 |
| | " | 20 | − 3.5 ± 1.5 |
| | | | Amplitude of after-discharge, microvolts |
| Thalamus | Saline | — | + 200 ± 190 |
| | Diph. | 20 | −1550 ± 650 |
| | Chlo. | 10 | −1690 ± 610 |
| | " | 40 | −1940 ± 540 |
| Cortex | Saline | — | − 50 ± 40 |
| | Diph. | 20 | −3400 ± 540 |
| | Chlo. | 40 | −1900 ± 490 |
| Hippocampus | Saline | — | + 790 ± 300 |
| | Diph. | 20 | − 290 ± 330 |
| | Chlo. | 40 | − 705 ± 220 |

* Figures show mean ± standard error of mean; 5 experiments per dose.

† Difference between mean pre-injection value and mean post-injection value.

Abbreviations: Chlo. = Chlordiazepoxide; Diph. = Diphenylhydantoin; Trim. = Trimethadione.

creased the threshold of the central lateral nucleus of the thalamus. At 400 mg/kg there was also a decrease in duration of response of this nucleus. Neither dose had any effect on medial dorsal nucleus, cortex or hippocampus.

*Diphenylhydantoin,* 10 mg/kg, decreased duration of response in cortex and hippocampus, but not in central lateral nucleus. At 20 mg/kg the drug raised the threshold of central lateral nucleus and cortex, and decreased duration and amplitude of response in all areas tested.

*Chlordiazepoxide,* 10 mg/kg, changed threshold, duration and amplitude of central lateral nucleus. At 40 mg/kg the drug also decreased the amplitude of response in cortex and hippocampus.

*Discussion.* Gastaut and Hunter(4) observed that the EEG response to photic stimulation in the cat was normally localized in the visual areas. Following injection of pentylenetetrazol, the response spread widely over thalamus and cortex. This "irradiated response" was selectively abolished by trimethadione. Since the irradiated response seemed to be identical to that produced by stimulation of intralaminar nuclei of the thalamus, the action of trimethadione was attributed to this area. Our results directly support this hypothesis.

Diphenylhydantoin differs clinically from trimethadione in being effective in grand mal rather than in petit mal epilepsy; in the laboratory it differs in lacking "any threshold-raising ability in animals, when minimal seizures or EEG signs of seizures are taken as the end point"(13). These authors suggest that diphenylhydantoin may act by "decreased responsiveness of the excited system" rather than by increased threshold. This suggestion accords with our finding that diphenylhydantoin at 10 mg/kg affected the duration of the after-discharge but not its threshold; at 20 mg/kg effects on threshold and amplitude were observed. Other workers in the cat observed that this drug reduced amplitude and duration of seizure discharges(14) and raised threshold and reduced duration of after-discharge(12). In the monkey this drug raised threshold for seizures(3,1).

Several authors have attempted to localize the action of diphenylhydantoin. The drug had no effect on convulsive movements in decerebrate cats, indicating that it acted on "nervous mechanisms higher than the mesencephalon"(7). Other studies in the cat showed that it did not influence the reticular activating system(8), while it did depress the cortex (14) and cortex and hippocampus(12). In the monkey, the drug depressed motor cortex, amygdala and anterior dorsal nucleus of thalamus(3), and motor cortex and hippocampus (1). These results are in general agreement with ours.

Chlordiazepoxide has been reported to show anti-convulsant activity in mouse(10), cat (11), monkey(2) and man(6,9). In the present study, this drug at low dosage resembled trimethadione in acting on the central lateral nucleus of the thalamus; at higher dosage it resembled diphenylhydantoin in depressing cortex and hippocampus.

*Summary.* Trimethadione, 100 mg/kg i.v., raised the threshold of the central lateral nucleus of the thalamus of the cat. The drug had no effect on medial dorsal nucleus, cortex or hippocampus. Diphenylhydantoin, 10 mg/kg, decreased duration of response in cortex and hippocampus, but not in thalamus. Chlordiazepoxide, 10 mg/kg, raised threshold and decreased duration and amplitude of response in central lateral nucleus.

We wish to thank Mrs. Mary Byrne for the histology and statistical analysis.

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## Reversibility of Reaction Between Human C'1 and Sensitized Sheep Erythrocytes.* (28177)

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Laporte and coworkers(1) prepared EA-gpC'$_1$, the complex between sensitized sheep erythrocytes (EA) and guinea pig (gp) C'1, by incubating EA with the midpiece fraction of hydrazine treated gp serum (R4). In extending this work to the human (hu) system a marked influence of temperature on dissociation of EA-huC'$_1$ was observed, which is described in this communication.

*Materials and methods.* Triethanolamine buffered saline (TBS)(2) containing 0.05% gelatin (Knox Intravenous) was used for all dilutions and washes. EA, prepared in the presence of EDTA(3), were washed and resuspended to a concentration of $5 \times 10^8$ cells/ml. All intermediate complexes prepared from EA were adjusted to this concentration unless otherwise noted. The complement reagents R1 and R4 were prepared according to the general methods described in Kabat and Mayer(4). Human R3 and the complex EA-huC'$_{1,4,2}$ were also prepared by established techniques(5). EA-huC'$_{1,4,2}$ was converted to EA-huC'$_{1,4}$ by thermal inactivation at 37°C for 45 minutes. C'1 was eluted from this complex by two 10 min extractions at 37°C with TBS containing 0.008 M trisodium ethylenediamine tetraacetic acid ($Na_3$-HEDTA)(1). The product, EAhuC'$_4$, was washed free of $Na_3$HEDTA before use.

The complex EA-huC'$_1$ was prepared by incubating one volume of R4 (usually a 1:10 dilution) with 2 volumes of EA at 37°C for 1 minute. Five volumes of TBS warmed to 37°C were added and the suspension centrifuged at room temperature for 1.5 min to minimize cooling *(vide infra)*. The sedimented cells were resuspended in TBS at 37°C, recentrifuged at room temperature for 1.5 min, resuspended in fresh TBS at 37°C and maintained at 37°C until used. Incubation of EA with R4 at 37°C for longer times than 1-3 min resulted in loss of EA-huC'$_1$ activity similar to that observed by Klein(6) in the gp system. Incubation at lower temperatures gave poor yields of EAhuC'$_1$. Tests for presence of C'1 activity bound to EA (EA-huC'$_1$, EA-huC'$_{1,4}$) were performed by mixing 1 ml of complex with 3 ml of a non-lytic dilution of R1 (usually 1:50) and incubating at 32°C for one hour (7).

Free C'1 was measured by adding test solution (1 ml) to packed EA-huC'$_4$ ($1 \times 10^9$) and incubating for 5 min at 37°C to allow formation of EA-huC'$_{1,4}$. The bound C'1 was then titrated as described above. Optical density of hemolysates was determined at 541 mμ.

*Results.* Initial observations on dissociation of C'1 were made using the complex EA-huC'$_{1,4}$. Aliquots (1 ml) were centrifuged at 0°C or 22°C for 1.5 min, washed with 5 ml portions of TBS at either 0°C or 37°C and tested for C'1 activity. Repeated washing at 0°C decreased the C'1 activity of the complex (Table I), whereas little or no loss of activity was observed after washing at 37°C.

Stability of EA-huC'$_1$ as a function of temperature was examined by dispensing 1 ml aliquots into tubes containing 5 ml of TBS maintained at 37°C, 22°C or 0°C. After incubating for 5 min at these temperatures

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