A Study of Convulsants Under the Influence of Reserpine and α-Benzoylamino-β-(3Pyridyl) Acrylic Acid Piperidide. (28118)

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Reserpine has been shown to facilitate the extensor-seizure response in mice to the convulsive action of pentylenetetrazol and certain other agents; this effect of reserpine, however, was absent in convulsions induced by strychnine(1,2). Alpha-benzylamino-β-(3 pyridyl) acrylic acid piperidide (BA3P) was found, on the other hand, to potentiate strychnine convulsions, yet it had an antagonistic action toward pentylenetetrazol seizures(3). These properties of reserpine and BA3P have been utilized to examine the convulsant properties of 19 compounds; the data are presented here.

Materials and methods. Male albino mice of Webster strain weighing 18-24 g were used. The test compounds were: strychnine nitrate; 5,7 - diphenyl - 1 - 3 - diazadamantan - 6 - ol (1757I.S.); hexahydro-2′-methylspiro[cyclohexane - 1,8′(6H) - oxazino[3,4 - A]pyrazine] (COP); picrotoxin; 1 - hydrazinophthalazine (hydralazine); 10-(2-dimethylaminopropyl)-9-acridone (DAPA); α-benzoylamino-β-(4-pyridyl) acrylic acid piperidide (BA4P); 4-methyl-4-ethylglutarimide (bemegride); α,α,β,β-tetramethyl succimide (PM-1090); pentylenetetrazol (Metrazol); nikethamide, bulbocapnine; caffeine; camphorimide; 1,5,5-trimethyl-2,4-dithiohydantoin (TMTH); N-isopropyl bicyclo (2,2,1)-5-heptene-2,3-dicarboximide (IDCI); 4,4,4-trichloro-3-hydroxy-N-(2,2,2-trichloro-1-hydroxyethyl) butyramide (THBA); hexahydro-1-isopropyl-2(3H)-benzimidazolone · H_2O (IPBI); and 17β-dimethylamino-5-androsten-3β-ol (DMAA).

To examine the facilitatory effect of reserpine on the extensor siezure response of mice, an aqueous solution of the convulsant was injected *via* the marginal tail vein at the rate of 0.05 ml per 10 seconds. The quantities of solution given to produce the initial clonic seizure, the subsequent tonic-extension of limbs, and death by respiratory failure were recorded(4). The concentration of a convulsant was so chosen that 0.1 to 0.15 ml and 0.3 to 0.5 ml would be required to produce the initial clonic and the subsequent extensor seizures respectively in control mice without reserpine pretreatment. A facilitatory effect of reserpine was considered to be present when the difference in volumes of solution taken to produce the initial clonic and the subsequent extensor seizures in reserpinized animals was less than 0.05 ml(5). In the case of bulbocapnine and nikethamide, only a few mice without reserpine pretreatment would respond with tonic extensor seizures by this technic of intravenous administration of maximally concentrated solutions of the 2 convulsants. The facilitatory influence of reserpine was then judged by a significant increase in the number of reserpinized animals in comparison with that of untreated controls, which responded with extensor siezures to intravenous infusion of bulbocapnine or nikethamide. Six compounds were not sufficiently soluble in water for intravenous injections. THBA, IDCI and IPBI were suspended in gum acacia solution and administered intraperitoneally; camphorimide, TMTH and DMAA were dissolved in 2% ascorbic acid and injected intramuscularly. The dosages of these compounds were submaximally convulsive quantities with which tonic extensor seizures could be produced in only a small percentage of mice without pretreatment with reserpine. With the same challenging doses of these convulsants, an increase in number of reserpinized animals having tonic extensor seizure, more than 50% of the untreated controls, was considered to be caused by the facilitating action of reserpine. Each convulsant was tested in 2 groups of 10 mice each with and without reserpine treatment. The values given in the tables are averages for 10 mice.

The influence of BA3P on response of mice to a convulsive agent was investigated by comparing the doses which would be required to produce convulsions in 50% of the BA3P pretreated mice and in 50% of untreated controls. No attempt was made here to dif-

TABLE I. Influence of Reserpine on Convulsions Induced by Various Compounds in Mice.

Compound	Injection, % or mg/kg	ml/20 g or No. responded / No. used: Untreated* I.C.	Untreated* T.E.	Treated I.C.	Treated T.E.	R.F.*
Strychnine	.005% i.v.	—	.34	—	.38	—
1757I.S.	.008% "	—	.32	—	.30	—
COP	.1 % "	—	.32	—	.18	+
BA4P	.5 % "	.29	.44	.25	.26	+
Picrotoxin	.15 % "	.29	.36	.34	.37	—
DAPA	.05 % "	.31	.38	.34	.39	—
Hydralazine	1.0 % "	.17	.32	.20	.29	—
Caffeine	1.0 % "	.24	.33	.18	.20	+
Bulbocapnine	1.5 % "	.10	0/10	.10	10/10	+
THBA	70 i.p.	10/10	1/10	10/10	9/10	+
Bemegride	.25 % i.v.	.11	.29	.10	.11	+
PM-1090	.1 % "	.15	.41	.16	.21	+
Camphorimide	40 i.m.	10/10	3/10	10/10	8/10	+
TMTH	6 "	10/10	5/10	10/10	10/10	+
Nikethamide	1.5 % i.v.	.10	3/10	.10	10/10	+
Metrazol	.5 % "	.16	.49	.16	.17	+
IDCI	60 i.p.	10/10	0/10	10/10	6/10	+
DMAA	40 i.m.	10/10	0/10	10/10	6/10	+
IPBI	120 i.p.	10/10	0/10	10/10	10/10	+

* See text. Seizure pattern: I.C. = initial clonic seizures; T.E. = tonic extensor seizures. Aqueous solutions in percent were given by intravenous infusion; other preparations in ascorbic acid or gum acacia solution were administered intramuscularly or intraperitoneally, respectively. ml/20 g = avg for 10 mice. R.F. = reserpine facilitation.

ferentiate the seizure patterns produced by each drug. The 50% convulsive dose of a compound was determined in 3 groups of 10 mice each at 3 graded dosages which would produce convulsions in 10 to 90% of the animals. The CD_{50} with standard errors was estimated by the linear relationship of probits to log-doses according to the graphic procedure of Miller and Tainter(6). The standard errors of a ratio of CD_{50}s were calculated from their individual standard errors in percentages(7). The water soluble compounds (or with addition of 2% ascorbic acid) were given by intramuscular injection, the insoluble ones by intraperitoneal injection.

BA3P as the hydrochloride (167 mg/kg of the base) dissolved in water and reserpine (4 mg/kg) in 5% ascorbic acid solution were administered intraperitoneally half an hour and 4 hours respectively before the animals were given the challenging dose of a convulsant.

Results and discussion. The test compounds in Tables I and II are listed according to seizure patterns they produce at highly convulsive dose levels in mice without reserpine pretreatment. At the top of the list are strychnine, 1757I.S., and COP, which cause predominantly tonic extensor siezures. Clonic convulsions only, on the other hand, are the effect of the last 2 compounds (IPBI and DMAA). With the other compounds listed the tonic extensor seizures are preceded by clonic convulsions.

The similarity and differences in convulsant properties of these compounds, as examined under the influence of reserpine and BA3P, were as follows: Both strychnine- and 1757I.S.-induced convulsions were unaffected by reserpine but were potentiated by BA3P. The convulsive action of 1757I.S. thus appears to be similar to that of strychnine; this confirms the observations of Longo *et al.*(8). The COP-induced seizure, which was indistinguishable from strychnine convulsion, was also potentiated by BA3P; COP differed from strychnine in that the effect of COP was augmented by reserpine. As in the case of COP, the BA4P induced convulsion was potentiated by BA3P, and the extensor component of the convulsion was facilitated by reserpine. The difference between COP and BA4P was that the latter produced clonic convulsions prior to the tonic extensor seizures, while the former did not.

TABLE II. CD_{50}s of Various Compounds in BA3P-Treated and Untreated Mice.

Compound	Untreated $CD_{50} \pm$ S.E. mg/kg	Treated $CD_{50} \pm$ S.E. mg/kg	Untreated / Treated	Effect*
Strychnine	.64 ± .06 i.m.	.16 ± .01 i.m.	4.13 ± .47	Potentiation
1757I.S.	2.92 ± .09 "	1.48 ± .07 "	1.97 ± .11	"
COP	3.85 ± .97 "	30.00 ± .84 "	1.28 ± .05	"
BA4P	77.00 ± 2.10 "	21.60 ± 1.50 "	3.57 ± .26	"
Picrotoxin	26.00 ± 1.30 "	14.50 ± 1.40 "	1.79 ± .20	"
DAPA	23.00 ± .50 "	22.70 ± .50 "	1.01 ± .03	No
Hydralazine	116.00 ± 2.90 "	164.00 ± 4.20 "	.71 ± .03	Suppression
Caffeine	350.00 ± 7.10 "	360.00 ± 5.80 "	.97 ± .03	No
Bulbocapnine	137.00 ± 5.70 "	146.00 ± 5.40 "	.93 ± .04	"
THBA	28.00 ± 2.00 i.p.	28.70 ± 1.70 i.p.	.98 ± .09	"
Bemegride	24.00 ± .70 i.m.	25.50 ± .50 i.m.	.94 ± .03	"
PM-1090	9.80 ± .20 "	11.00 ± .40 "	.88 ± .04	Suppression
Camphorimide	24.50 ± 1.00 "	28.00 ± 1.20 "	.87 ± .05	"
TMTH	3.18 ± .16 i.p.	3.88 ± .17 i.p.	.82 ± .06	"
Nikethamide	300.00 ±11.20 i.m.	385.00 ± 7.90 i.m.	.78 ± .03	"
Metrazol	54.50 ± 2.50 "	74.00 ± 2.70 "	.74 ± .04	"
IDCI	40.30 ± 2.60 i.p.	78.00 ± 3.00 i.p.	.52 ± .04	"
DMAA	21.50 ± 1.30 "	25.00 ± 1.60 "	.86 ± .08	"
IPBI	69.00 ± 2.50 "	68.00 ± 2.20 "	1.02 ± .05	No

* As judged by CD_{50} ratio of "untreated" to "treated": > 1 = potentiation; < 1 = suppression; "P" values = .01 to .05.

The tonic extensor seizures induced by picrotoxin, DAPA and hydralazine were not affected by reserpine. Picrotoxin convulsion was potentiated by BA3P, but DAPA convulsion was not; in contrast, hydralazine convulsion was suppressed by it. The extensor seizure response of mice to the convulsive action of the next 12 compounds on the list was facilitated by reserpine, including those which did not produce extensor seizures in animals without reserpine pretreatment. There was no potentiation between these convulsants and BA3P. A suppressive effect of BA3P was obtained in convulsions induced by PM-1090, camphorimide, TMTH, nikethamide, Metrazol, IDCI and DMAA, but was not seen in convulsions induced by caffeine, bulbocapnine, THBA, bemegride and IPBI.

Under the influence of reserpine or/and BA3P, it is thus possible to reveal some of the differences in convulsant properties of the various compounds which produce similar seizure patterns in mice. For instance, COP would be considered to be a strychnine-like agent were it not for the fact that reserpine potentiates the convulsive effect of the former but not that of the latter drug. The difference in convulsant properties between picrotoxin, Metrazol, and bemegride is of practical interest since these 3 drugs are used for the management of barbiturate-induced narcosis. Is picrotoxin, Metrazol or bemegride a more efficacious analeptic drug in combating barbiturate depression? Very likely, other analeptic compounds will be introduced into the clinic in the future. Such a study as presented here may be useful for characterization and assessment of potential therapeutic agents.

Summary. The influence of reserpine and α-benzoylamino-β-(3 pyridyl) acrylic acid piperidide (BA3P) upon the response of mice to the convulsive action of 19 drugs has been investigated. Reserpine was found to facilitate tonic extensor seizures induced by COP, BA4P, caffeine, bulbocapnine, THBA, bemegride, PM-1090, camphorimide, TMTH, nikethamide, Metrazol, IDCI, DMAA and IPBI. It did not affect the tonic extensor seizures induced by strychnine, 1757I.S., picrotoxin, DAPA, and hydralazine. BA3P produced (a) potentiation, (b) suppression, or (c) no affect toward convulsions induced respectively by (a) strychnine, 1757I.S., COP, BA4P, and picrotoxin; (b) hydralazine, PM-1090, camphorimide, TMTH, nikethamide, Metrazol, IDCI, and DMAA; and (c) DAPA, caffeine, bulbocapnine, THBA, bemegride and IPBI.

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Received December 3, 1962. P.S.E.B.M., 1963, v112.

Effect of Triethylenemelamine (TEM) on Endocrine System of the Male Rat.* (28119)

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In the search for anti-fertility drugs that can be used in the male, it becomes important to determine whether there is interference with the male endocrine system. Several investigators have demonstrated that the radiomimetic agent, triethylenemelamine (TEM), interferes with fertility in male rodents(1,2,3,4). Although the data presented by Steinberger *et al.*(4) showed some depression in weights of accessory sex glands following treatment with TEM, these authors felt there was no indication of interference with androgen level since the body weights of the animals were also lowered. The consensus has been that other agents which produce damage to the seminiferous epithelium, such as X-ray, do not damage the more resistant Leydig cells. Studies conducted in this laboratory have shown, however, that within 12 days after whole-body exposure to X-ray there is an androgen depression, even in the face of normal-appearing Leydig cells(5). Since TEM is classified as a radiomimetic agent, it seemed pertinent to investigate more thoroughly its possible effects on the male endocrine system.

Materials and methods. Mature, male Wistar rats, 16 weeks of age (350 to 400 g), were used in these studies. TEM was administered once daily for 5 days in a dose of 0.2 mg/kg. TEM was made up fresh in physiological saline on the day of the first injection and was stored at 4°C during the 5-day injection period. TEM was administered by the intraperitoneal route in volumes of 1 ml/kg body weight.

Since both the weight of the dorsolateral prostate and its capacity to take up administered Zn^{65} have been shown to be under androgen(6), or ICSH control(7,8), these two parameters were used to assess possible endocrine disturbances following TEM treatment. On the 5th, 15th or 19th day following termination of a 5-day treatment with TEM, rats were injected intracardially with Zn^{65}† (0.04 μc/g). A group of non-TEM injected rats of the same age also received Zn^{65}. Twenty-four hours later, the rats were sacrificed; and dorsolateral prostates were dissected as described previously(9). Tissues were placed on tared aluminum foil, dried, weighed and their radioactivity determined in a large, well-type scintillation detector.

The effect of administration of hormones was also studied in TEM-treated rats. During the 5 days following TEM administration, rats were injected daily with 200 μg of testosterone propionate‡ (subcutaneous in 0.2

* Supported in part by a research grant from U. S. Atomic Energy Commission and in part by a U. S. Public Health Service research grant from Nat. Inst. of Arthritis and Metab. Dis.

† Zn^{65} was purchased from Union Carbide Nuclear Co. as $Zn^{65}Cl_2$ in HCl solution with a specific activity of approximately 1000 mc/g. The solution was diluted with physiological saline containing sufficient NaOH to neutralize some of the acid present compatible with solubility.