solvent removal. With these precautions, limits of detection of bromide were approximately 1×10^{-2} mcg. which is far less sensitive than spectrofluorometric and gas chromatographic methods for certain drugs. With longer activation times perhaps the sensitivity could be increased but costs of analyses would also increase and, perhaps, background problems.

REFERENCE

(1) Steim, J. M., and Benson, A. A., *Anal. Biochem.*, **9**, 21(1964).

Keyphrases

Neutron activation, derivative—analysis
Chlorpromazine—test compound
Aspirin, bromine derivatives—test compounds
Paper chromatography—separation, degradation products
TLC—separation, degradation products

Effects of Some Adrenergic Agents on Low Frequency Electroshock Seizures

By PENG NAM YEOH and HAROLD H. WOLF

The effect of several adrenergic agents on low frequency electroshock seizures was studied in normal, reserpine and α-mmT pretreated mice. Seizure threshold was elevated by pronethalol and propranolol and reduced by phenoxybenzamine and isoproterenol. Both reserpine and α-mmT antagonized the threshold altering activity of pronethalol and phenoxybenzamine. Reserpine plus pronethalol or propranolol completely abolished the central stimulation induced by isoproterenol. A central adrenergic system involving antagonistic types of receptors appears to be involved.

It has been shown that β-adrenergic blocking agents possess anticonvulsant activity when evaluated by maximal electroshock and chemoshock techniques (1, 2). Preliminary studies in these laboratories (unpublished) indicate that the β-adrenergic blocking agents pronethalol and propranolol also reduce seizure susceptibility in audiogenic seizure susceptible mice, whereas phenoxybenzamine, an α-adrenergic blocking agent, enhances this phenomenon.

It was the objective of this study to determine whether such drug effects reflect general changes in the level of brain excitability which could be readily quantitated by measuring alterations in low frequency electroshock seizure threshold (l.f. EST). Increasing evidence indicates that endogenous brain amines may play an important role in the expression of seizure states (3–6). The experiments were designed to facilitate an evaluation of the relationship between the adrenergic drug activity described and the existing level of endogenous brain amines.

EXPERIMENTAL

Adult, male, albino mice of a random bred Swiss strain (Maxfield Animal Supply, Cincinnati, Ohio) ranging from 16 to 28 Gm. were used as experimental animals. They were housed in groups of 9 to 11 animals in plastic cages (26 × 15 × 12 cm.) with wire mesh or perforated metal tops. Except during periods of experimentation, they were maintained on Purina laboratory chow and given free access to tap water.

Pronethalol [(2-isopropylamino)-1-(2-naphthyl) ethanol hydrochloride] and propranolol [(1-isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride] were employed as β-adrenergic blocking agents and phenoxybenzamine hydrochloride as an α-adrenergic blocking agent. The catecholamine depleting agents used were reserpine (2 mg./Kg., 24-hr. pretreatment) and α-methyl-metatyrosine (α-mmT; 400 mg./Kg., 24-hr. pretreatment).

All drugs were given intraperitoneally as solutions in distilled water except pronethalol which was administered in 0.2% methylcellulose.[1] They were prepared so that 10 ml./Kg. contained the required dose. Requisite volumes of saline or methylcellulose (0.2%) were administered to control animals. In studies where two or more drugs were employed in sequence, the control animals received only the drug (or drugs) used for pretreatment. Except for fluorometric assays, all experiments were conducted between 4 and 11 p.m. at 23–25°.

Neurotoxicity Studies—The method of Weaver and Miya (7) with slight modifications was employed to obtain the time of peak effect and the dose of each of the adrenergic agents which produced overt evidence of neurological toxicity in 50% of mice (TD_{50}). Mice were placed on a rolling bar, 2.5 cm. in diameter, rotating at 6 r.p.m. An animal was considered to be unaffected if it could stay on the bar continuously for 1 min. in any one of three consecutive trials. Data obtained in this test were statistically analyzed by the method of Litchfield and Wilcoxon (8). In all further experiments (unless otherwise stated) the dose of all adrenergic agents used was $^1/_2$ TD_{50}.

Low Frequency Electroshock Seizure Threshold (l.f. EST) Test—A Grass stimulator (model S4G, Grass Instrument Co., Quincy, Mass.) was used to apply a unidirectional current (6 pulses per second, 0.2 msec. duration, 3 sec. stimulus duration) through corneal electrodes (9). Mice were shocked at various intensities according to the staircase method of Finney (10). The presence of a "stunning" response (at least 3 sec. of immobility) or minimal clonus was con-

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[1] Methocel. Dow Chemical Co., Midland, Mich.

TABLE I—NEUROTOXICITY OF SOME ADRENERGIC BLOCKING AGENTS

Drug	Time of Peak Effect, min.	Toxic Dose$_{50}$, mg./Kg.
Pronethalol	5	52.0 (35.9–75.4)[a]
Propranolol	15	59.5 (53.1–66.6)
Phenoxybenzamine	60	74.0 (64.9–84.4)

[a] The figures within parentheses represent 95% fiducial limits.

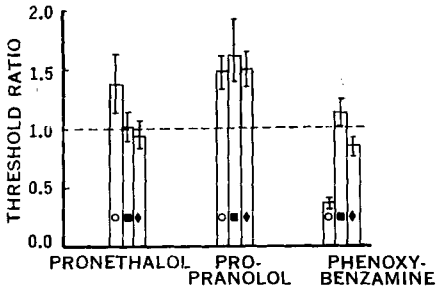

Fig. 1—Effect of some adrenergic blocking agents on l.f. EST. Key: ○, *drug* per se; ■, *reserpine + drug;* ◆, *α-mmT + drug.*

sidered indicative of a seizure. Groups of at least 24 mice each were administered $^1/_2$ TD_{50} of the various adrenergic agents and subjected to the l.f. EST test at the time of peak neurotoxicity. Control groups were always tested concurrently with drug-treated animals. The l.f. EST was determined as previously described (11). The threshold ratio (threshold of drug group/threshold of control group) was calculated and the 95% confidence limits for this ratio determined by the method of Litchfield and Wilcoxon (8).

Catecholamine Assays—The amount of depletion of brain catecholamines (norepinephrine and epinephrine) produced by reserpine and α-mmT (under experimental conditions where the depleting agents, *per se*, do not alter seizure threshold) was determined. Fluorometric assays of whole brains (3 brains per sample) were conducted according to a modified method of Anton and Sayre (12). Fluorescence was read using an Aminco-Bowman spectrophotofluorometer with photomultiplier tube IP21. The excitation–emission wavelengths for norepinephrine at pH 7.0 and for epinephrine at pH 2.0 were (395–505) and (410–520), respectively. The level of brain catecholamines (mcg./Gm. wet tissue) in reserpine and α-mmT pretreated mice was compared statistically to that of control animals by means of a *t* test.

RESULTS

A summary of the results obtained in the neurotoxicity studies is presented in Table I.

The effects of $^1/_2$ TD_{50} of the various adrenergic blocking agents employed on l.f. EST can be seen in Fig. 1. In this and Fig. 2, the vertical bracketed lines represent 95% confidence intervals for the calculated ratios and any line which does not cross 1.0 indicates that the treatment employed had a significant ($p < 0.05$) effect on convulsive threshold.

Thus it can be calculated that pronethalol and

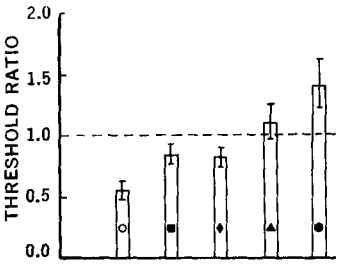

Fig. 2—Effect of isoproterenol on l.f. EST. Key: ○, *isoproterenol* per se; ■, *reserpine + isoproterenol;* ◆, *α-mmT + isoproterenol;* ▲, *reserpine + pronethalol + isoproterenol;* ●, *reserpine + propranolol + isoproterenol.*

propranolol elevate seizure threshold by 37% and 47%, respectively, whereas phenoxybenzamine lowers it by 65% when compared to saline-treated controls. Pretreatment with either reserpine or α-mmT antagonizes most of the central effects of pronethalol and phenoxybenzamine, but not that of propranolol.

No significant change in l.f. EST was demonstrated in control mice treated with either reserpine (2 mg./Kg., 24-hr. pretreatment) or α-mmT (400 mg./Kg., 24-hr. pretreatment). However, under these same experimental conditions, significant depletion of brain catecholamines was demonstrated. An examination of the results of the fluorometric assay of brain catecholamines indicated that the dose of reserpine employed produces 85.8% and 63.2% depletion of norepinephrine and epinephrine, respectively, whereas that of α-mmT induces 70.0% depletion of norepinephrine and 31.3% depletion of epinephrine.

DISCUSSION

The results of this investigation indicate that the anticonvulsant effects of pronethalol and propranolol do extend to low frequency electroshock seizures. Furthermore, phenoxybenzamine appears to lower markedly the threshold for such seizures. While the central activity seen with propranolol does not seem to be related to the existing brain level of endogenous catecholamines, that observed following treatment with either pronethalol or phenoxybenzamine is significantly altered in animals whose catecholamines have been partially depleted by reserpine or α-mmT.

Since essentially opposite effects on l.f. EST were produced by the α- *versus* β-adrenergic blocking agents examined, it was decided to investigate the central effects of an agent known to stimulate selective adrenergic receptors in the periphery. For this purpose the β-receptor stimulant isoproterenol was chosen and the same experimental procedure outlined above was followed. The TD_{50} for isoproterenol was found to be 470 (401.7–549.9) mg./Kg. with a time of peak effect of 10 min. The effect of isoproterenol on l.f. EST may be seen in Fig. 2.

Isoproterenol *per se* markedly lowers seizure threshold at a dose of $^1/_2$ TD_{50}. Pretreatment with either reserpine or α-mmT significantly reduces (but does not abolish) this effect. Finally, this same dose of isoproterenol does not induce any evident central stimulation in animals pretreated

with reserpine and a β-adrenergic blocking agent (pronethalol or propranolol). In fact, it can be seen that the propranolol plus reserpine pretreated mice have elevated seizure thresholds following the administration of isoproterenol.

The results of this preliminary study suggest the presence of central adrenergic receptors which may be involved in the expression of l.f. EST. This concept is supported by the previous work of Swinyard *et al.* (3, 4), who demonstrated that the threshold for both chemically and electrically induced seizures in mice can be altered by prior administration of epinephrine and norepinephrine. Moreover, Baldessarini and Kopin (13) have recently reported that a marked release of ^{3}H-norepinephrine from rat brain occurs in response to electrical stimulation and Scudder *et al.* (14) have demonstrated the existence of a positive correlation between the level of endogenous brain amines and maximal electroshock seizure latency in mice.

The failure to observe consistently significant alterations in l.f. EST when reserpine or α-mmT were given *per se* may reflect incomplete amine depletion, lack of inhibition of amine biosynthesis (in the case of reserpine), or production of active metabolites from α-mmT, *e.g.*, *m*-hydroxyamphetamine, metaraminol, or *m*-methoxyamphetamine (15). Nevertheless, the results observed with the adrenergic agents employed seem to imply that threshold for low frequency electroshock seizures may be markedly influenced by the degree of drug-induced differential stimulation applied to opposing central adrenergic receptors. In order to substantiate this hypothesis, additional data of a similar nature must be collected for a variety of adrenergic drugs which are known to interact specifically with α and β receptors. Furthermore, the work must be conducted in animals that have been subjected to a more selective inhibition of brain amine biosynthesis than that reported above. Such studies are currently in progress.

REFERENCES

(1) Murmann, W., Almirante, L., and Saccani-Guelfi, M., *J. Pharm. Pharmacol.*, **18**, 317(1966).
(2) Leszkovszky, G., and Tardos, L., *ibid.*, **17**, 518(1965).
(3) Swinyard, E. A., Radharkrishnan, N., and Goodman, L. S., *J. Pharmacol. Exptl. Therap.*, **138**, 337(1962).
(4) Swinyard, E. A., Boson, F. C. B., and Goodman, L. S., *ibid.*, **144**, 52(1964).
(5) Pfeifer, A. K., and Galambos, E., *Biochem. Pharmacol.*, **14**, 37(1965).
(6) Pfeifer, A. K., and Galambos, E., *J. Pharm. Pharmacol.*, **19**, 400(1967).
(7) Weaver, J. E., and Miya, T. S., *J. Pharm. Sci.*, **50**, 910(1961).
(8) Litchfield, J., and Wilcoxon, F., *J. Pharmacol. Exptl. Therap.*, **96**, 99(1949).
(9) Spiegel, E., *J. Lab. Clin. Med.*, **22**, 1274(1937).
(10) Finney, D. J., "Experimental Design and its Statistical Basis," The University of Chicago Press, Chicago, Ill., 1955, p. 120.
(11) Swinyard, E. A., Chin, L., and Fingl, E., *Science*, **125**, 739(1957).
(12) Anton, A. H., and Sayre, D. F., *J. Pharmacol. Exptl. Therap.*, **138**, 36(1962).
(13) Baldessarini, R. J., and Kopin, I. J., *Science*, **152**, 1630(1966).
(14) Scudder, C. L., Karczmar, A. G., Everett, G. M., Gibson, J. E., and Rifkin, M., *Intern. J. Neuropharmacol.*, **5**, 343(1966).
(15) Van Rossum, J. M., *Psychopharmacologia*, **4**, 271 (1963).

Keyphrases

Adrenergic agent
α-adrenergic blocking agents
β-adrenergic blocking agents
Catecholamine depleting agents
Electroshock, low frequency—seizure threshold effect
Neurotoxicity—adrenergic agents
Fluorometry—brain tissue analysis

Ion-Exchange Resins and Cinchona Alkaloids I. Exchange Equilibria

By S. S. KANHERE, R. S. SHAH, and S. L. BAFNA

The exchange equilibria of four cinchona alkaloid sulfates with sulfonic acid cation-exchange resins of different degrees of cross-linking and particle size have been studied and the results are discussed.

THE AVAILABLE ion-exchange studies with cinchona alkaloids (1–21) do not seem to cover detailed physicochemical studies and hence it should be of interest to undertake such work. This paper summarizes the study of exchange equilibria of four cinchona alkaloid sulfates with styrene divinylbenzene copolymer-based sulfonic acid cation-exchange resins (in hydrogen form) of different degrees of cross-linking (percent nominal divinylbenzene content) and particle size.

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EXPERIMENTAL

Resins (22) and chemicals (23) were from the samples used in earlier work. The stock solution of the alkaloid sulfate (as $Q_2H_2SO_4 \cdot nH_2O$, where Q denotes the alkaloid base) was prepared in distilled water and its concentration in gram equivalents (half the molecular weight of the alkaloid sulfate) per liter was evaluated by sulfate estimation (as barium sulfate).

To study the exchange equilibria of the alkaloid sulfates with the resins, weighed amounts of air-dried resins were placed in contact with suitable volumes of the aqueous sulfate solution of known concentration, in well-stoppered flasks, with frequent shaking at room temperature (~30°). Preliminary work was carried out to find the approximate time within which equilibrium was attained. After sufficiently more time than this (about 4 to 35 days, depending