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POTENTIATION AND ANTAGONISM OF BIOGENIC AMINES[1, 2]

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ABSTRACT

RAUZZINO, FELIX J. AND JOSEPH SEIFTER: Potentiation and antagonism of biogenic amines. J. Pharmacol. Exp. Therap. **157**: 143–148, 1967. The 24- to 48-hr-old chick lacks an effective blood-brain barrier and thereby provides a useful subject for evaluating the direct action of drugs on the central nervous system. Dose-response curves and their slopes were obtained by intravenously administering biogenic amines or γ-aminobutyric acid either alone or in combination. When these curves for lethargy were compared in chicks, the depressant biogenic amines fell into three groups. When two amines of group A (epinephrine and tryptamine) were administered in combination, potentiation occurred, but, when an amine of group A (epinephrine) was administered in combination with an amine of group B (5-hydroxytryptamine, 5-hydroxytryptophan, *l*-tryptophan or histamine), increased potentiation occurred. The interaction between 5-hydroxytryptamine, and epinephrine was remarkable in that there was an apparent supersensitivity. The interaction between a group A (epinephrine) and a group C compound (γ-aminobutyric acid) resulted in insignificant potentiation. Epinephrine antagonized the excitement induced by 5-hydroxy-N,N-dimethyltryptamine. Other depressant biogenic amines such as 5-hydroxytryptamine, *l*-tryptophan and 5-hydroxytryptophan did not antagonize 5-hydroxy-N,N-dimethyltryptamine. The characteristic behavioral response of the depressant amines could not be induced in older chickens because of the presence of an effective blood-brain barrier.

Catecholamines, indoleamines and certain other amino compounds administered systemically often fail to penetrate into brain tissue because of the existence of an effective blood-brain barrier. Injection into the cerebral circulation (Leimdorfer, 1950), into the cerebral ventricles (Feldberg and Sherwood, 1954) or directly into brain tissue (Haley and McCormick, 1957) is not accepted as a physiologic procedure for circumventing the blood-brain barrier. Neither is application by iontophoresis (Krnjevic and Phillis, 1963) nor injury of brain tissue (Purpura *et al.*, 1958) a more satisfactory procedure. Administration of precursor amino acids that are presumably degraded to the active amines (Udenfriend *et al.*, 1956; Hess *et al.*, 1959) is not a decisive method for blood-brain barrier studies. More recently it has been recognized that most mammals and birds do not have an effective blood-brain barrier at birth (Waelsch, 1955; Marley and Key, 1963), and that the behavioral responses of young animals to systemically administered biogenic amines may be quite different from those of older ones.

It is well established in young chicks that epinephrine and norepinephrine produce behavioral (Clymer and Seifter, 1947; Zaimis, 1960) and electrocortical sleep (Key and Marley, 1962). Similarly, 5-hydroxytryptamine (5-HT), histamine, γ-aminobutyric acid (GABA) and certain precursor amino

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acids of these and their analogs also produce behavioral and electrocortical sleep (Seifter *et al.*, 1963; Kramer and Seifter, 1964, 1966; Scholes, 1965; Spooner and Winters, 1965). If duration of sleep is used as the measure of reaction, then combinations of subeffective doses of biogenic amines could interact to provide quantitatively different responses. Comparisons of such effects were, therefore, undertaken.

METHODS. More than 900 sex-linked hybrid cockerels (24–48 hr old), obtained from Hall Brothers Hatchery, Wallingford, Conn., and 80 male chickens (3–4 months old) were used. The drugs were dissolved in distilled water or 0.9% NaCl. These were administered intravenously either into the saphenous (chicks) or alar (chickens) vein by means of a 30-gauge needle and a 0.5-ml syringe. Maximal volume injected never exceeded 0.4 ml. For studying the effects of two agents, appropriate combinations were prepared and the mixtures were administered immediately as single injections. Controls were similarly injected with an equivalent volume of distilled water or 0.9% NaCl.

Since it is known that environmental variables affect bird behavior (Key and Marley, 1962; Dewhurst and Marley, 1965), all experiments were performed in an illuminated, noise-free room maintained at 21–23°C and 65 to 69% humidity. To avoid the effects of group reaction, each bird was injected and isolated: chicks in 10- by 10- by 6-inch lidless cardboard boxes and chickens in 24- by 24- by 36-inch cages. Only one bird was treated at a time and all observations were made by one of the authors (F.R.). Upon recovery, the bird was killed.

Duration of the lethargy complex produced by each amine was measured from the time of onset until complete recovery when treated chicks appeared to be as alert and active as untreated ones.

The following drugs were employed: *l*-epinephrine bitartrate, *l*-norepinephrine-*d*-bitartrate monohydrate, tryptamine hydrochloride, 5-hydroxytryptamine creatine sulfate, histamine dihydrochloride, *l*-tryptophan hydrochloride, *d*-tryptophan hydrochloride, *dl*-tryptophan hydrochloride, 5-hydroxytryptophan hydrochloride, γ-aminobutyric acid, *dl*-amphetamine sulfate, 5-hydroxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine. All doses were calculated as the free base. The results were analyzed statistically by the multiple regression technique (Ostle, 1958).

RESULTS. *Behavioral effects in chicks.* The behavioral responses induced by the depressant amines were similar. These have been classified by us as the lethargy complex and consisted of a characteristic posture corresponding to natural roosting, eyelid closure and immobility. Figure 1A shows the mean duration times of lethargy induced by depressant amines expressed in minutes. It also shows that the duration of lethargy is dose-related.[6]

Two dimethyl derivatives of tryptamine, 5-hydroxy-N,N-dimethyltryptamine (5-HDMT) and N,N-dimethyltryptamine, produced amphetamine-like responses (table 1) as reported by Seifter *et al.* (1963). The excitement, twittering and chirping were intermittent and of shorter duration.

Log dose-response curves. Log dose-response curves were constructed for each depressant amine. The contours of the curves of figure 1A appeared similar. For comparative purposes, activity was conveniently defined as 10 min of lethargy in 100% of the chicks. Epinephrine, norepinephrine, 5-hydroxytryptamine and tryptamine were active below 6 mg of base per kg. Histamine, 5-hydroxytryptophan (5-HTP) and *l*-tryptophan (*l*-TP) were active in the range of 9.8 to 13.0 mg of base per kg. For the least effective agents, *dl*-TP and γ-aminobutyric acid, estimates for 10-min duration required extending the curves and extrapolating. *dl*-TP acted at a dose above 136 mg of base per kg, thereby indicating that the racemic form had one-twelfth (not the anticipated one-half) the effectiveness of the *l*-form. GABA was the weakest and acted only at doses in excess of 3 g of base per kg.

Potentiating effects of combinations of a subeffective dose of epinephrine with biogenic amines in chicks. The effective dose of epinephrine for inducing lethargy in all chicks for a mean duration of 10 min was 1.0 mg of base per kg; one-thirtieth of this dose (0.03 mg of base per kg) was completely ineffective (fig. 1A). When this subeffective dose of epinephrine was combined with subeffective doses of various biogenic amines (fig. 1B), conversion to effectiveness occurred as mani-

[6] All injections were made using distilled water as a vehicle because the amines were more readily soluble in water than in 0.9% NaCl. Comparative range-finding studies using both vehicles showed that the results were essentially identical.

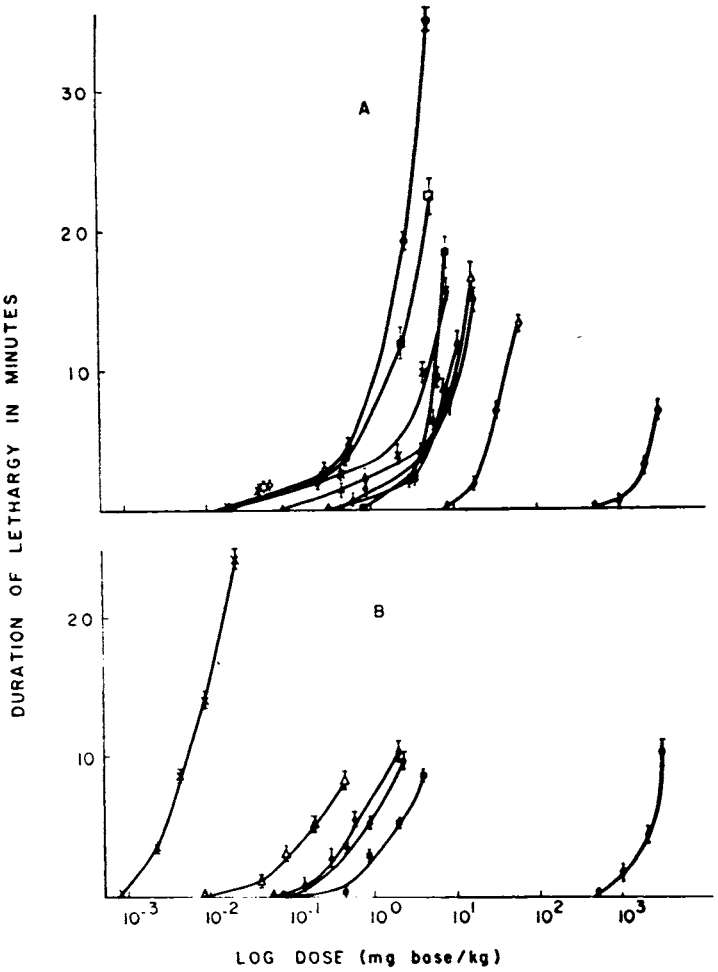

FIG. 1. A, comparison of the log dose-response curves for duration of lethargy in chicks. B, comparison of the log dose-response curves for the potentiating effects of combinations of a subeffective dose of epinephrine with various biogenic amines in chicks. Key to symbols: ○, epinephrine; □, norepinephrine; ■, tryptamine; ×, 5-HT; △, 5-HTP; ●, *l*-tryptophan; ▲, histamine; ◇, *dl*-tryptophan; ◆, GABA. Each point and standard deviation represent 6 experiments or more.

fested by change from no lethargy to mean durations of lethargy lasting 8.7 to 24.2 min. The log dose-response curves in figure 1B show that combinations of epinephrine with 5-HT, 5-HTP, histamine or *l*-TP gave curves that resembled those of figure 1A but differed significantly in that they were shifted to the left ($P < .001$). Subeffective doses of epinephrine shifted the log dose-response curve of tryptamine to the left ($P < .05$) and failed to change the effects of GABA.

Subeffective doses of 5-HT in combination with a subeffective dose of epinephrine became markedly potent. This pronounced shift to the left with a somewhat steeper slope demonstrated that the combined treatment was 607 times more effective than 5-HT alone and 53 times more effective than epinephrine alone.

Antagonism of epinephrine and some indoles against 5-HDMT-induced excitement in chicks. Administration of 1.0 mg of base per kg of 5-HDMT produced chirping, drooped wings and excitement lasting 72 min; 5.5 mg of base per kg of epinephrine produced lethargy for 34 min. Combinations of these amines prevented both the typical effect of epinephrine and the 5-HDMT-induced re-

TABLE 1

Behavioral effects of amphetamine, 5-HDMT and N,N-dimethyltryptamine

Compound	Dose	No. of Chicks	Effect					Mortality
			Chirping	Twittering	Drooped wings	Excitement	Panting	
	mg base/kg							(%)
Amphetamine	1.0	6	0	6	6	6	6	0
	5.0	6	0	6	6	6	6	0
	10.0	6	0	6	6	6	6	0
	20.0	6	0	6	6	6	6	0
5-HDMT	0.1	6	6	0	6	6	6	0
	0.5	6	6	0	6	6	6	0
	1.0	6	6	0	6	6	6	0
	5.0	6	0	6	6	6	6	0
N,N-dimethyl-tryptamine	1.0	6	6	0	6	0	6	0
	5.0	6	6	0	6	6	6	0
	10.0	6	6	2	6	6	6	0
	20.0	6	6	3	6	6	6	0

TABLE 2

Effect of various amines on 5-HDMT-induced excitement

Compounds Administered Simultaneously	No. of Chicks	Effect			Mortality
		Chirping	Drooped Wings	Excitement	
mg base/kg					(%)
H_2O + 5-HDMT (1.0)	6	6	6	6	0
Epinephrine (5.5) + 5-HDMT (1.0)	6	0[a]	6	0[a]	0
5-HT (10.0) + 5-HDMT (1.0)	6	6[b]	6	6	0
l-Tryptophan (17.0) + 5-HDMT (1.0)	6	6[c]	6	6	0
5-HTP (17.2) + 5-HDMT (1.0)	6	6[c]	6	6	0

[a] Thirty minutes after initial injection, chicks appear as 5-HDMT controls.

[b] Chirping markedly reduced for 10 min.

[c] Chirping slightly reduced for 5 min.

sponse for at least 30 min (table 2). During this time, the chicks appeared similar to the untreated controls. After this interval, the antagonism due to epinephrine diminished so that the 5-HDMT excitement emerged. On the other hand, 5-HT, *l*-TP and 5-HTP antagonized only the increased rate of normal chirping.

Behavioral effects in older chickens. Nonlethal doses of either epinephrine (0.03–2.7 mg of base per kg) or 5-HT (0.02–0.4 mg of base per kg) produced only defecation and ataxia (table 3). Lethal doses of either epinephrine (5.5–11 mg of base per kg) or 5-HT (2.2–4.4 mg of base per kg) produced side position and clonic convulsions before death. Amphetamine, 5-HDMT and N,N-dimethyltryptamine produced excitement of short duration. The 5-HDMT-treated birds became aggressive and attacked on provocation. Histamine and GABA produced no visible symptoms at the doses administered.

Discussion. These experiments were designed to demonstrate the possible interaction of certain amines on the central nervous system before and after complete formation of a blood-brain barrier. In our studies, behavioral changes in intact birds indicated direct action of the test substances on the brain. Since the brain is a heterogeneous cellular complex, the observation that similar functional activity occurs as a result of drug treatment does not necessarily imply similar or identical sites of action.

Examination of our log dose plots showed curves that were apparently parallel, differing only in horizonal position. However, close examination suggested the possibility of three groups (A, B, C) which were more similar to each other than they were individually (fig. 1A): A, *l*-epinephrine, *l*-norepinephrine and tryptamine; B, 5-HTP and *l*-TP; C, GABA and *dl*-TP. Whether or not this subtle tend-

TABLE 3

Behavioral effects of various amines in the chicken

Compound	Dose	No. of Chickens	Effect							Mortality
			Side position	Chirping	Drooped wings	Excitement	Aggression	Tremors	Convulsions	
	mg base/kg									(%)
Epinephrine	0.03	4								0
	0.05	4								0
	0.3	4								0
	0.5	4								0
	2.7	4								0
	5.5	4	4						1	25
	11.0	4	4						4	100
5-HT	0.02	4								0
	0.04	4								0
	0.2	4								0
	0.4	4								0
	2.2	4							2	50
	4.4	4							4	100
Amphetamine	1.0	4								0
	5.0	4		4	4	4		4		0
	10.0	4		4	4	4		4		0
5-HDMT	1.0	4			4	4	4	4		0
N,N-dimethyl-tryptamine	10.0	4			4	4		4		0
Histamine	6.0	4								0
GABA	1000.0	4								0

ency to grouping is of sufficient significance to warrant such classification requires additional exploration.

Figure 1B shows that epinephrine produced a different degree of potentiation with each agonist. Based on the horizontal shifts of the log dose-response curves, the agonists may be ranked: 5-HT; 5-HTP, histamine and *l*-TP; tryptamine and GABA.

Epinephrine produced the greatest degree of potentiation with 5-HT and any small increment in the dose of 5-HT resulted in a very rapid rate of change in duration of response. This could be assessed as a supersensitivity of the test animal. A different situation exists in mammals such as cats and dogs. Brodie *et al.* (1959) hypothesized that a catecholamine such as norepinephrine produces different pharmacologic actions from those produced by 5-HT. They assumed that these two amines modulate opposing systems in the brain, thereby implying that contrasting central effects are exerted. Epinephrine potentiates agonists such as 5-HTP, histamine and *l*-TP so that their activity is 8 to 15 times greater than that of the agonists alone. The agonist group consisting of tryptamine and GABA was weakly effective, as shown by the slight shift to the left in the position of the dose-response curves.

Antagonism between compounds exerting contrasting effects could also be demonstrated. The epinephrine-induced lethargy and the 5-HDMT-induced excitement were absent when both amines were administered simultaneously. In these studies, the doses were sufficient to prevent the characteristic behavioral patterns for either amine from becoming evident. When these amines interacted to maintain a counterbalance, the behavioral response of neither epinephrine nor 5-HDMT predominated. An imbalance occurred when the behavioral effects of epinephrine became dissipated. This was shown by the shorter duration of restraint exerted by epinephrine with the subsequent emerging of excitement induced by 5-HDMT.

On the basis of these results and those of

other workers (Waelsch, 1955; Marley and Key, 1963), it appears that the changes that we observed in older birds, as compared to chicks, were related to maturation of the blood-brain barrier. The depressant amines did not penetrate into the brain of older chickens and were inactive. The excitant amines apparently permeated to a lesser degree than in younger chicks and induced an attenuated reaction.

It is possible by analysis of the dose-response curves (Mackay, 1966) to postulate whether or not common sites are affected by the various amines. We recognize that the consideration of different receptor populations is speculative in the absence of specific agonist-antagonist studies. It is true in isolated systems that sympathomimetic amines of different potencies may act on one and the same receptor. Although the responses are some function of the fraction of receptors occupied by the agonist (Clark, 1933), this function is not necessarily a linear one (Schild, 1947). The principles established for isolated tissues or organs may or may not apply to more complex systems.

REFERENCES

Brodie, B. B., Spector, S. and Shore, P. A.: Interaction of the drugs with norepinephrine in the brain. Pharmacol. Rev. **11**: 548–564, 1959.

Clark, A. J.: Kinetics of cell-drug reactions. *In* Mode of Action of Drugs on Cells, p. 61, The Williams & Wilkins Co., Baltimore, 1933.

Clymer, N. V. and Seifter, J.: A method of screening sympathomimetic amines for stimulant action on the cerebrum. J. Pharmacol. Exp. Therap. **89**: 149–152, 1947.

Dewhurst, W. G. and Marley, E.: Methods for quantifying behaviour and cerebral electrical activity and the effects of drugs under controlled conditions. Brit. J. Pharmacol. Chemotherap. **25**: 671–681, 1965.

Feldberg, W. and Sherwood, S. L.: Injection of drugs into the lateral ventricle of the cat. J. Physiol. (London) **123**: 148–167, 1954.

Haley, T. J. and McCormick, W. G.: Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Brit. J. Pharmacol. Chemotherap. **12**: 12–15, 1957.

Hess, S. M., Redfield, B. G. and Udenfriend, S.: The effect of monamine oxidase inhibitors and tryptophan on the tryptamine content of animal tissues and urine. J. Pharmacol. Exp. Therap. **127**: 178–181, 1959.

Key, B. J. and Marley, E.: The effect on the sympathomimetic amines on behaviour and electrocortical activity of the chicken. Electroencephalogr. Clin. Neurophysiol. **14**: 90–105, 1962.

Kramer, S. Z. and Seifter, J.: Electrocortical and behavioral effects of centrally active drugs in chicks. Federation Proc. **23**: 148, 1964.

Kramer, S. Z. and Seifter, J.: The effects of GABA and biogenic amines on behavior and brain electrical activity in chicks. Life Sci. **5**: 527–535, 1966.

Krnjevic, K. and Phillis, J. W.: Actions of certain amines on cerebral cortical neurons. Brit. J. Pharmacol. Chemotherap. **20**: 471–490, 1963.

Leimdorfer, A.: The action of sympathomimetic amines on the central nervous system and the blood sugar. Mechanism of action. J. Pharmacol. Exp. Therap. **98**: 62–71, 1950.

Mackay, D.: General analysis of the receptor-drug interaction. Brit. J. Pharmacol. Chemotherap. **26**: 9–16, 1966.

Marley, E. and Key, B. J.: Maturation of the electrocorticogram and behaviour in the kitten and guinea-pig and the effect of some sympathomimetic amines. Electroencephalogr. Clin. Neurophysiol. **15**: 620–636, 1963.

Ostle, B.: Statistics in Research, pp. 117–162, Iowa State College Press, Ames, 1958.

Purpura, D. P., Girado, M., Smith, T. G. and Gomez, J. A.: Synaptic effects of systemic γ-aminobutyric acid in cortical regions of increased vascular permeability. Proc. Soc. Exp. Biol. Med. **97**: 348–353, 1958.

Schild, H. O.: pA, a new scale for the measurement of drug antagonism. Brit. J. Pharmacol. Chemotherap. **2**: 189–206, 1947.

Scholes, N. W.: Effects of parenterally administered γ-aminobutyric acid on the general behavior of the young chick. Life Sci. **4**: 1945–1949, 1965.

Seifter, J., Rauzzino, F. and Kramer, S. Z.: The effect of some indoles in chicks. Pharmacologist **5**: 246, 1963.

Spooner, C. E. and Winters, W. D.: Evidence for a direct action of monamine on the chick central nervous system. Experientia **21**: 256–258, 1965.

Udenfriend, S., Bogdanski, D. F. and Weissbach, H.: Increase in tissue serotonin by administration of its precursor, 5-hydroxy-tryptophane. Federation Proc. **15**: 493, 1956.

Waelsch, H.: Blood-brain barrier and gas exchange. *In* Biochemistry of the Developing Nervous System, pp. 187–207, Academic Press, New York, 1955.

Zaimis, E.: Synthesis of catechol amines in depleted brain. *In* Ciba Foundation Symposium on Adrenergic Mechanisms, ed. by J. R. Vane, G. E. W. Wolstenholme and M. O'Connor, pp. 562–566, Little, Brown & Co., Boston, 1960.