Bioassay of Endogenous Acetylcholine Released by Acetylcholine Releasers

Keyphrases ☐ Acetylcholine, endogenous—bioassay ☐ Ileum, guinea pig—endogenous acetylcholine bioassay

Sir:

Cumulative, indirect evidences have shown that some cholinergic agents act wholly or partly through the release of endogenous acetylcholine (ACh) from nerve terminals (1–3). However, because bioassay procedures do not differentiate between the ACh released from the nerve terminals and the ACh-releasing agents, it is extremely difficult to provide direct evidence for the release of ACh by cholinergic agents.

It has been reported that nerve tissue in the guinea pig ileum is irreversibly damaged by maintaining the ileum in Tyrode's solution without oxygen at 2° for 20 hr. However, after this treatment, the smooth muscle is still responsive to ACh (4). This cooling technique has been combined with the superfusion technique to produce a method for the direct assay of endogenous ACh in the presence of releasing agents.

Guinea pigs weighing 300 to 500 g. were stunned by a blow on the head and the terminal portion of the ileum, approximately 3 cm. in length, was excised. The ileum was threaded at both ends and was superfused with oxygenated Tyrode's solution (NaCl, 8.0; KCl, 0.2; $CaCl_2$, 0.2; $MgCl_2$, 0.1; NaH_2PO_4, 0.05; $NaHCO_3$, 1.0; dextrose, 2.0 g./l.) at 10°. The superfusion technique was essentially the same as that described by Gaddum (5). The rate of flow of superfusion fluid was 3–4 ml./min. and was maintained by a motor pump (Holter, type RD 45). Drug solutions, tissue extracts, or incubation mixtures of drugs and synaptic vesicles were injected into the superfusion stream in volumes of not more than 0.1 ml. An initial tension of 1.0 g. was placed on the tissue and the subsequent tension, developed by muscle contraction, was measured in grams and recorded on a recorder (Offner Dynograph, type RS). The cooling technique described above, was used except the ileum was cooled for 24 rather than 20 hr.

Figures 1 and 2 show the dose-response curves for ACh and nicotine before and after the cooling treatment. The cooled ileum was about two times more sensitive to ACh than the normal tissue (Fig. 1) but was inactive to nicotine (Fig. 2). Therefore, this superfused cooled ileum preparation can be used to assay released endogenous ACh present in mixtures of tissue extracts and releasing agents or in incubation mixtures of synaptic vesicles and such releasing agents as nicotine, carbachol, choline, tetramethylammonium (TMA), *etc.* It is a rapid, sensitive, and accurate method capable of detecting as little as 0.0025–0.005 mcg. ACh per injection without eserinizing the preparation.

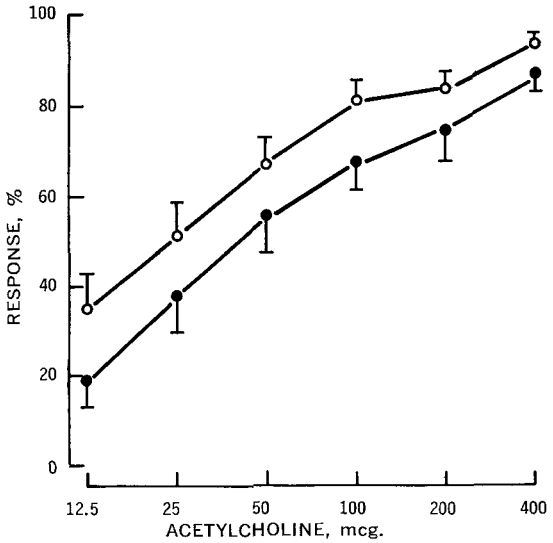

Figure 1—*Dose-response curves of acetylcholine on superfused guinea pig ileum. Key:* ●, *control responses of normal ileum;* ○, *responses of cooled ileum. Each point is the mean of five experiments and the bars represent standard errors.*

The nerve tissue in the cooled ileum did not appear to be completely damaged after cooling since the cooled

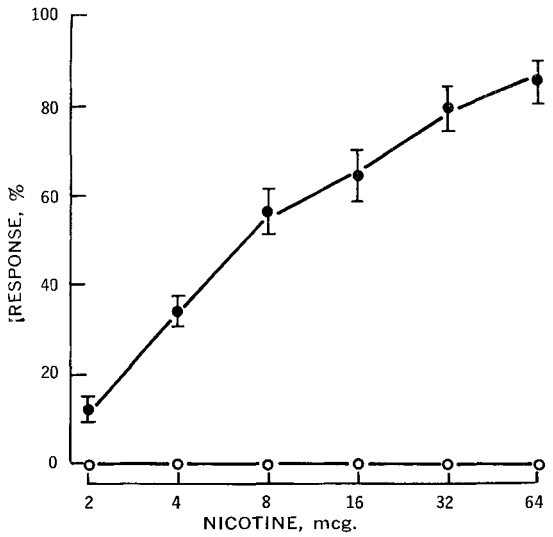

Figure 2—*Dose-response curves of nicotine on superfused cooled guinea pig ileum. Key:* ●, *control responses of normal ileum;* ○, *responses of cooled ileum. Each point is the mean of five experiments and the bars represent standard errors.*

ileum was still responsive to initial injections of nicotine, choline, carbachol, and TMA. However, responses to the maximum doses of these agents gradually decreased; after repeated injections of maximum doses, the responses disappeared or were markedly reduced whereas the responses to ACh were not altered. It is important, therefore, to deplete the minute amount of endogenous ACh in the nerve tissue of the cooled ileum with ACh releasers before the bioassay is performed. The technique has been used to determine ACh released from synaptic vesicles by some cholinergic agents (6).

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Investigation of Adrenergic Beta-Receptor Blockade and Mescaline-Induced Bradycardia

Keyphrases ☐ Mescaline-induced bradycardia—adrenergic blockade effect ☐ Isoproterenol activity—mescaline effect ☐ Ethylnorepinephrine activity—mescaline effect

Sir:

Mescaline-induced bradycardia was first observed in frogs and cats by Grace in 1934 (1). This activity was not affected by vagotomy or by pretreatment with atropine in either *in vitro* or *in vivo* preparations. In 1955 Speck reported mescaline-induced bradycardia in rats, and suggested a possible mescaline competition for epinephrine receptors (2). Recently, in the course of studies concerned with the design and standardization of an autonomic-cardiovascular screen in dogs, mescaline-induced bradycardia was documented again (3). As shown in Table I, bradycardia was seen in the dosage range of 6.4–25.6 mg./kg. Dramatic changes in heart rate and pressor response to serial intravenous *l*-epinephrine challenge injections were also documented with dosages as low as 0.4 mg./kg. The decrease in the epinephrine challenge heart rate suggested that the resting bradycardia might be due to adrenergic beta-receptor blockade. The increased epinephrine pressor response also seemed to imply this through restricted adrenergic alpha-receptor-induced vasoconstriction. This postulation is supported by the report that another hallucinogen, lysergic acid diethylamide, possesses adrenergic beta-receptor-blocking activity in rabbits (4). In the present study, mescaline[1] was tested for adrenergic beta-blocking activity using specialized, qualitative *in vitro* and *in vivo* testing procedures.

Table I—Effects of Cumulative Intravenous Mescaline on the Resting Heart Rate and Epinephrine Challenge in an Intact Urethan-Anesthetized Dog

Cumulative Dosage, mg./kg.	Mean Percent Change from Pre-Mescaline Control Cycles[a]		
	Resting Heart Rate	Epinephrine Heart Rate	Epinephrine Pressor Response
0.1–0.2	−1	−18	−6
0.4–3.2	0	−36	+56
6.4–25.6	−26[b]	−52	+97
51.2 (lethal)	—	−38	+98

[a] During the control cycles (3), the mean stabilized pre-mescaline heart rate was 154/min. The mean pre-mescaline pressor response to a standardized injection of 4 mcg./kg. of epinephrine was a 44-mm. Hg elevation from the resting level of 134 mm. Hg. The heart rate slowed to 116/min. durnig the peak of the pressor response. The dog was not vagotomized. [b] A similar degree of bradycardia was induced by cumulative intravenous dosages of 0.08–1.28 mg./kg. of propranolol in a similar dog preparation.

The specific *in vitro* procedure of Levy and Tozzi (5) was used in which beta blocking agents are known to antagonize isoproterenol-induced relaxation of the spontaneously contracting rat uterus. The perfusate was Locke's solution, aerated with 95% O_2 and 5% CO_2 at 37.5°. Mescaline showed no isoproterenol[2] antagonism at tenfold increments between 0.005–5.0-mcg./ml. bath concentrations (Fig. 1). The 5.0-mcg./ml. mescaline bath concentration produced significant increases in uterine contraction rate and contracture without truly antagonizing isoproterenol (Fig. 1). These results, which were confirmed in another tissue preparation, indicated that mescaline, itself, does not possess adrenergic beta-blocking activity at concentrations without intrinsic activity. Propranolol[3] served as a standard reference beta blocker and was shown to be effective at bath concentrations of 0.1 mcg./ml. in antagonizing

[1] Mescaline HCl (lot D3303) was obtained from Mann Research Laboratories, Inc., New York, N. Y. All text references are expressed in terms of the salt.

[2] The HCl salt was obtained from the Special Chemicals Dept., Winthrop Laboratories, New York, N. Y. All text references are in terms of the salt (5).

[3] Propranolol (Inderal) was kindly supplied by the Medical Dept., Ayerst Laboratories, New York, N. Y.