Hexamethonium potentiation of noradrenaline-induced contractions in the dilatator pupillae of the cat

SIR,—The dilatator pupillae has hitherto been thought to be supplied by adrenergic nerves only. Selective denervations and staining methods, however, have now conclusively proved that cholinergic, parasympathetic fibres form a well-developed network in the dilatator of mice (Ehinger & Sporrong, 1966), rats (Ehinger & Falck, 1965, 1966; Ehinger & Sporrong, 1967) and cats (Ehinger, 1967). The acetylcholinesterase-containing nerve fibres to the dilatator of a number of other species (Laties & Jacobowitz, 1964, 1966; Lukáš, 1964; Ehinger, 1966) most probably also represent a cholinergic, parasympathetic supply. In rats and mice, it has further been shown that the adrenergic and cholinergic fibres run closely intertwined in the vegetative nerve network (Ehinger & Falck, 1965, 1966; Ehinger & Sporrong, 1967). In a recent investigation of the function of these cholinergic nerve fibres of the dilatator (Ehinger, Falck & Persson, 1967) it was found that atropine potentiates contractions induced by noradrenaline or high frequency (50 c/sec) electrical stimulation in the cat dilatator. The phenomenon could readily be explained on the basis of the newly-detected cholinergic fibres, which relax the muscle. As the cholinergic and adrenergic fibres presumably run very close to each other and as structures of synaptic character between nerve fibres have been observed in electron microscopical work on the colon (Hagen, 1966; Dr. E. van der Zypen, personal communication), and moreover, as no ganglia occur in the cat iris, it was of interest to study the effect of the pure ganglionic inhibitor (see Nádor, 1960) hexamethonium. Cat iris dilatators were mounted and tested in an organ bath as described previously (Ehinger & others, 1967). The noradrenaline concentration was usually 5 μg/ml. The electrical stimulation was 50 c/sec, 1–3 V. The fact that this stimulation produces no contractions in sympathetically denervated muscle shows that it acts via the adrenergic nerves (Schaeppi & Koella, 1964; Ehinger & others, 1967). The contractions induced were always markedly submaximal.

As is seen from Tables 1 and 2, hexamethonium (10 μg/ml) enhanced the noradrenaline-induced contractions of the dilatator, but not contractions resulting from the electrical stimulation. Selective parasympathectomy (Ehinger, 1967) abolished the enhancing effect of hexamethonium.

TABLE 1. EFFECT OF HEXAMETHONIUM (10 μg/ml) ON THE RESPONSE TO STIMULATION WITH NORADRENALINE

Normal			Ciliary ganglionectomy		
Contraction force, mg		Difference %	Contraction force, mg		Difference %
Before drug	After drug		Before drug	After drug	
32	47	+47	41	44	+ 7
24	41	+71	58	40	−31
18	38	+111	10	9	−10
24	34	+42	26	25	− 4
23	28	+22	21	20	− 5
34	64	+88	—	—	—
22	28	+27	—	—	Mean − 8·6
13	25	+92	—	—	s.e.m. ± 6·3
15	16	+ 7	—	—	—
41	58	+41	—	—	—
28	32	+14	—	—	—
—	—	Mean +51·0	—	—	—
—	—	s.e.m. ±10·4	—	—	—
Significance of difference from zero		P < 0·001			Not significant

Significance of difference between normal and denervated muscle: P < 0·01.

TABLE 2. EFFECT OF HEXAMETHONIUM (10 μg/ml) ON THE RESPONSE TO ELECTRICAL STIMULATION

Normal muscles		
Contraction force, mg		
Before drug	After drug	Difference %
22	24	+ 9
23	28	+ 8
28	29	+ 4
31	33	+ 7
58	58	0
38	26	−32
52	37	−29
16	21	+31
16	21	+31
35	42	+20
60	54	−10
37	36	− 3
26	28	+ 8
64	66	+ 3
44	43	− 2
46	44	− 4
54	48	−11
		Mean + 1·8
		s.e.m. ± 4·2

Hexamethonium is known to enhance the response of blood vessels to adrenaline and noradrenaline (see Hilton, 1962; Vitolina & Melzobs, 1964). It has been claimed that the effect is not a result of changes on the adrenergic receptor in the muscle (Vitolina & Melzobs, 1964). This is supported by the failure to record any enhancement of electrically-induced contractions; such an enhancement could be expected if the receptors had become sensitized to the release of noradrenaline from the nerve terminals. Hilton (1962) suggested that the effect was due to a shift in the position of the dose-response curve. Such a shift could well be due to the disappearance of an inhibitory system. In the dilatator, the cholinergic parasympathetic fibres have an inhibitory function (Ehinger & others, 1967) and it seems probable that hexamethonium decreases the function of these fibres. This could be effected either by lowering the transmitter release from the cholinergic fibres, or by lowering their sensitivity to stimulation with noradrenaline. There are reasons for presuming that cholinergic neurons possess receptors sensitive to noradrenaline both at the perikarya (see review by Norberg & Sjöqvist, 1966) and at the terminals (Leaders, 1963). If the effect should be due to a decrease in transmitter release, the response to electrical stimulation could be expected to be potentiated in the same way as the response to noradrenaline. This was not so; therefore it seems possible that hexamethonium exerts its effect by decreasing the sensitivity of the nerve fibre to stimulation with noradrenaline.

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Effect of tyramine on the atrium and the papillary muscle of the immunosympathectomized rat

SIR,—Burn & Rand (1958) proposed that tyramine exerted its sympathomimetic effects through the release of endogenous noradrenaline. This hypothesis is now generally accepted (Trendelenburg, 1963; Muscholl, 1966), although evidence for a direct action of tyramine has also been reported (Luduena, 1963; Varma & Benfey, 1963; Varma, Gillis & Benfey, 1964; Zaimis, 1965; Krzanowski & Woodbury, 1966). Most experiments on the mode of action of tyramine have been made after depleting noradrenaline stores by reserpine or by surgical denervation. Since immunosympathectomy can produce almost complete destruction of the peripheral sympathetic nervous system (Levi-Montalcini & Angeletti, 1962; Zaimis, 1965; Iversen, Glowinski & Axelrod, 1966), we examined the effect of tyramine on the myocardium of immunosympathectomized rats.

Immunosympathectomy was produced by subcutaneous injection of 0·2 ml of 61,000 anti-units/ml of bovine anti-serum to nerve-growth factor (kindly supplied by Dr. R. K. Richards, Abbot Laboratories, Chicago) in Sprague-Dawley rats within 24 hr of birth. The effectiveness of this treatment in producing immunosympathectomy was described by Iversen & others (1966) and confirmed by us (Varma, 1967) and also during the present experiments. Rats were used approximately 3 months after birth. A group of normal and immunosympathectomized rats were also injected subcutaneously with 1 mg/kg of reserpine one day before the experiment. Each rat was killed by a blow on the head and the heart rapidly excised. Atria were removed, freed of ventricular tissue and set up in a 100 ml organ bath containing Krebs-Henseleit solution at 37° and aerated with a mixture of oxygen 95% and carbon dioxide 5%. Spontaneous contractions were recorded by a Grass force-displacement transducer on a Gilson polygraph. Tension on the atria was adjusted to give maximum contraction. This was approximately 0·5 g. Papillary muscle was removed from the left ventricle and set up in a separate 100 ml organ bath under identical conditions. The muscle was stimulated by square wave pulses of 5 msec duration at 1 c/sec and supramaximal voltage. A Tektronix stimulator was used. Both preparations were allowed to stabilize for at least 1 hr during which period the bath fluid was changed several times. Cumulative concentration-response curves to tyramine were determined. Preparations were then washed repeatedly for 1 hr after which cumulative concentration-response curves to noradrenaline were determined. Initial concentration of tyramine hydrochloride was 0·01 μg/ml and that of noradrenaline bitartrate monohydrate 0·001 μg/ml. Concentrations were increased by a factor of about 3 and the next highest concentration was