Induction of rhythmicity in a normally quiescent smooth muscle

Bozler (1948) subdivided smooth muscle preparations into two types, single unit which has a low electrical resistance between adjacent cells, propagated electrical activity within the tissue, spontaneous rhythmicity and myogenic contraction, and multiunit which lacks the above properties. Somlyo & Somlyo (1968) suggested that smooth muscle may exhibit various shades of single or multiunit behaviour.

In the present communication we report the multiunit property of the cat spleen capsular smooth muscle and its conversion to the single unit type by pharmacological agents.

Spleen capsular smooth muscle preparations (Bose & Innes, 1972) were suspended in Krebs-Henseleit solution (37°) which was bubbled with 5% carbon dioxide in oxygen. A resting tension of 1 g was maintained. Noradrenaline produced an increase in isometric tension (Fig. 1A) or isotonic shortening but in 40 experiments in neither case was any rhythmic component seen. The unstimulated spleen strips were quiescent. Rapid stretching of the strips ($n = 20$ in 5 cats) with the help of an electromagnetic solenoid to 125% of their resting length never caused rebound contraction whether the preparation was under the influence of noradrenaline or otherwise (Fig. 2A). These observations suggested that the capsular strip normally behaved like other multiunit muscles (Burnstock & Prosser, 1960). This is consistent with the ultrastructural evidence showing a large amount of connective tissue between smooth muscle cells (Fillenz, 1970; unpublished observations).

In 10 experiments, cocaine (10^{-5} to 3×10^{-5} g ml^{-1}) potentiated responses to noradrenaline within 10 min. The tension response to noradrenaline instead of consisting of an initial fast phase and a subsequent slower phase now had a more prominent slow phase on which were superimposed additional rhythmic oscillations. These responses were seen with as little as 10^{-8} g ml^{-1} of noradrenaline. As the concentration of the agonist was increased, the frequency of the superimposed rhythmic contractions also increased until at 10^{-7} to 3×10^{-7} g ml^{-1} the oscillations became fused (Fig. 1B). Similar oscillations were also observed when another α-adrenoceptor stimulant, phenylephrine (10^{-5} to 10^{-4} g ml^{-1}) was used in the presence of cocaine. In 3 experiments cocaine (3×10^{-5} g ml^{-1}) itself caused a slight increase in tension along with oscillations. This was most likely due to release of endogenous catecholamines because similar results were not observed in spleen from cats treated with reserpine (1 mg kg^{-1}, 24 h before experiment). However, in such reserpine-

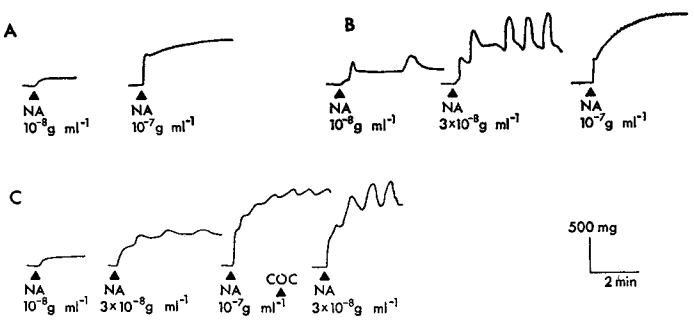

FIG. 1. Induction of rhythmicity in cat spleen capsule. Effects of increasing concentrations (10^{-8}, 3×10^{-8} and 10^{-7} g ml^{-1}) of noradrenaline (NA) in A, a normal strip; in B, a strip treated with cocaine (COC; 10^{-5} g ml^{-1}); in C, a strip obtained from a reserpine-treated cat. Note enhancement by cocaine (COC; 10^{-5} g ml^{-1}) of rhythmicity due to NA (3×10^{-8} g ml^{-1}).

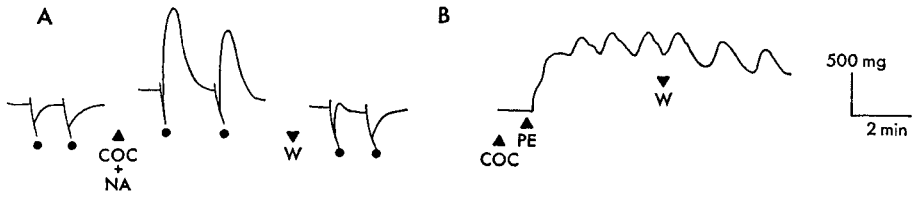

FIG. 2. A. Muscle was rapidly stretched and released (at ●). Note the myogenic response (upward deflection following ●) in the presence of cocaine (COC; 10^{-5} g ml^{-1}) and noradrenaline (NA; 10^{-8} g ml^{-1}) and its abolition after wash (▼).

B. Effect of lowering level of bathing medium (at ▼) on the rhythmic contractions induced by cocaine (COC; 10^{-5} g ml^{-1}) and phenylephrine (PE; 2×10^{-5} g ml^{-1}).

treated spleen strips, noradrenaline (10^{-8} to 3×10^{-7} g ml^{-1}) produced oscillations in 10 experiments the magnitude of which was further increased by cocaine (10^{-5} g ml^{-1}) (Fig. 1C).

Similar results were obtained in 6 experiments on spleen strips from cats treated with 6-hyroxydopamine (35 mg kg^{-1}, i.v. 24 h before experiment) to destroy peripheral adrenergic nerve endings (Tranzer & Thoenen, 1968).

In 3 experiments, tetrodotoxin (5×10^{-6} g ml^{-1}) was found not to abolish rhythmic oscillations due to the combined effects of cocaine and noradrenaline, suggesting that the oscillations were not neurogenic but myogenic (Kuriyama, Osa & Toida, 1966). Enhancement of myogenic reactivity was also evidenced by the ability of spleen strips treated with cocaine and noradrenaline to respond to rapid stretching with a substantial after-contraction in each of 30 attempts. The strips no longer responded to rapid stretch after noradrenaline and cocaine were washed out (Fig. 2A).

To test whether automaticity in the splenic smooth muscle was associated with conduction of activity from one part of the strip to another, the following experiment was done. Rhythmic contractions were induced with a combination of phenylephrine (2×10^{-5} g ml) and cocaine (10^{-5} g ml^{-1}) in 6 pairs of strips from 6 cats. After the automaticity and basal tension were stable, the bathing medium around one of each pair of muscles was lowered to make contact with only one half of the strip leaving the top half of the strip exposed to warm, moist air. The other strip served as a control. If the pacemaker cells initiating the rhythmic contractions existed in the lower half of the strip (bathed by the medium), and if this portion of the muscle was capable of conducting the excitatory wave of depolarization arising from the pacemakers, then the upper half of the strip exposed to air should also be automatic, and the total magnitude of rhythmic contraction should remain unchanged. On the other hand, if the conduction failed, the amplitude of rhythmic contraction should be reduced approximately by half. Finally, if the pacemaker cells were in the region of the strip exposed to air, the rhythmic contractions should stop completely (due to destruction of the limited amount of agonist in the extracellular space by monoamine oxidase). This would occur in spite of the ability of the lower half of the muscle to conduct impulses. Thus a decrease or abolition of rhythmicity in these experiments would not rule out single unit behaviour of the muscle, but unchanged rhythmicity would strongly suggest this type of behaviour. When the level of the medium surrounding the automatic strips was lowered, the basal active tension invariably decreased gradually. However, no definite conclusion could be drawn from the effects on rhythmicity; in 2 out of 6 experiments, the amplitude of rhythmic contractions was maintained (Fig. 2B) suggesting conduction of excitation, in 1 experiment it was diminished and in 3 experiments the rhythmicity was abolished.

The occurrence of myogenic reactivity to stretch and oscillations in the presence of cocaine, reserpine or 6-hydroxydopamine suggests that, regardless of the primary

site of action, the ultimate effect is an alteration in the property of the muscle cell. Both reserpine (Shore, 1962) and 6-hydroxydopamine (Tranzer & Thoenen, 1968) impair adrenergic neuronal function by causing depletion of noradrenaline stores and chemical sympathectomy respectively. Cocaine on the other hand impairs neuronal reuptake of catecholamines (Iversen, 1963). The exact relation of the above actions of these drugs with their ability to produce rhythmicity on the one hand and postjunctional, unspecific supersensitivity (Hudgins & Fleming, 1966; Kasuya & Goto, 1968; Davidson, 1970) is not clear. The possibility of the nerves mediating a controlling influence over postjunctional sites is attractive. Fillenz (1970) reported the presence of some tight junctions and in addition we have observed gap junctions and peg and socket type of interdigitations between adjacent smooth muscle cells in the spleen (unpublished observations). These structures are commonly associated with single unit types of smooth muscle that usually exhibit rhythmicity (Dewey & Barr, 1962). Absence of rhythmicity in the normal spleen indicates the functional inadequacy of such low resistance junctions in the normal spleen strips. It is equally possible that these histological junctions may be affected by drugs that induce rhythmic behaviour or the drug concerned may be inducing pacemaker activity in some cells, or both. The ultimate proof of single unit behaviour lies in electrophysiological studies.

It is interesting to note that the guinea-pig vas deferens, which is normally quiescent, can also be made to contract rhythmically when exposed to cocaine, procaine, lignocaine, piperoxan, thymoxamine or mepyramine (Cliff, 1968). This phenomenon was also seen most strikingly in the denervated preparation. Our results with the spleen capsule indicate that pharmacological modification of pacemaker activity and/or electrical coupling between smooth muscle cells is indeed possible in more than one tissue and it also suggests that presence of adrenergic nerve endings inhibit such myogenic activity in the normal situation.

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