Biphasic responses to guanosyl nucleotides in two smooth muscle preparations

The recent discovery that acetylcholine induces rapid increases in guanosine 3',5'-monophosphate (3',5' GMP) in brain, heart (Kuo, Lee & others, 1972) and smooth muscle (Lee, Kuo & Greengard, 1972) suggested that exogenous 3',5' GMP might mimic the effects of acetylcholine. We have examined the effects of 3',5' GMP and its derivative, N^6, 2-*O*-dibutyryl 3',5' GMP (dibutyryl 3',5' GMP), on the guinea-pig ileum and spirally cut trachea of the guinea-pig (Constantine, 1965).

The ileum and trachea were removed from female guinea-pigs (Hartley strain, 250–300 g) and suspended in a 10 ml organ bath containing Tyrode solution at 37° and allowed to equilibrate for 1 h. Contractions and relaxations were recorded isometrically using a pen recorder.

Low concentrations of 3',5' GMP (7·5–15 μg ml^{-1} threshold dose range for 8 preparations out of 14) and dibutyryl 3',5' GMP (5–10 μg ml^{-1} threshold dose range for 10 preparations out of 14) produced increases in tension with increasing doses in both tissues (e.g. Fig. 1A and B are representative for the dibutyryl derivative). No response was elicited in the remaining tissues. Contractions produced by both compounds on the ileum were maximal within 30–45 s but contractions of the tracheal strip developed more slowly (5 min for maximum response). This corresponds with the 60 s and 3 min delay for maximal responses to submaximal doses of acetylcholine (1 μg ml^{-1}) applied to the ileum and trachea respectively.

The contractions by exogeneous 3',5' GMP were abolished by atropine (1·0 μg ml^{-1}). in all 6 tracheal strips and all 4 ileal preparations. Eserine salicylate (1·0 μg ml^{-1}) given 5 min before dibutyryl 3',5' GMP potentiated the response of the low dose of 3',5' GMP in 6 tracheal strips from two to three fold. At this dose level eserine produces a slowly developing prolonged contraction (5–7 min) confirming the observations of Carlyle (1963). Consequently eserine and dibutyryl 3',5' GMP were given together to avoid the delayed contractile effect of eserine.

Sub-threshold concentrations of dibutyryl 3',5' GMP ($<$5 μg ml^{-1}) did not potentiate acetylcholine responses (0·25–0·5 μg ml^{-1}) when administered 60 s before, or at the same time as, the acetylcholine dose in either tissue. Likewise, sub-threshold doses of acetylcholine ($<$0·05 μg ml^{-1}) did not potentiate the contractile response to dibutyryl 3',5' GMP when given together with the nucleotide.

40–85 μg ml^{-1} 3',5' GMP (in all 7 preparations examined) and 30–55 μg ml^{-1} dibutyryl 3',5' GMP generally elicited no response on the ileum (e.g. Fig. 1A). Two preparations out of the ten examined produced a small contraction to dibutyryl 3',5' GMP followed by relaxation. Both 3',5' GMP (45–85 μg ml^{-1}, in all 6 preparations) and dibutyryl 3',5' GMP (30–80 μg ml^{-1}, in all 7 preparations) had no visible effect on the tracheal strip (e.g. Fig. 1B).

Higher doses of both drugs on both preparations produced dose-related relaxations which were maximal after approximately 30 s for the ileum (Fig. 1A) and 7 min for the trachea (Fig. 1B). The contractile response to the GMP nucleotides did not occur in all preparations although relaxation with higher doses was produced in every preparation. The relaxations produced by 3',5' GMP were not affected by propranolol in any of the tissues (2 μg ml^{-1}; 6 tracheal and 4 ileal preparations).

Guanosine 5'-monophosphoric acid (5' GMP) in doses $>$7 μg ml^{-1} produced relaxation of both the tracheal and ileal preparations (5 experiments with each tissue). This relaxation was not affected by propranolol (2 μg ml^{-1}). Guanosine (1–200 μg ml^{-1}) did not produce relaxation or contraction of the trachea (5 preparations).

The observation that intracellular levels of 3',5' GMP are elevated by acetylcholine (Kuo & others, 1972; Lee & others, 1972) has suggested that this cyclic nucleotide

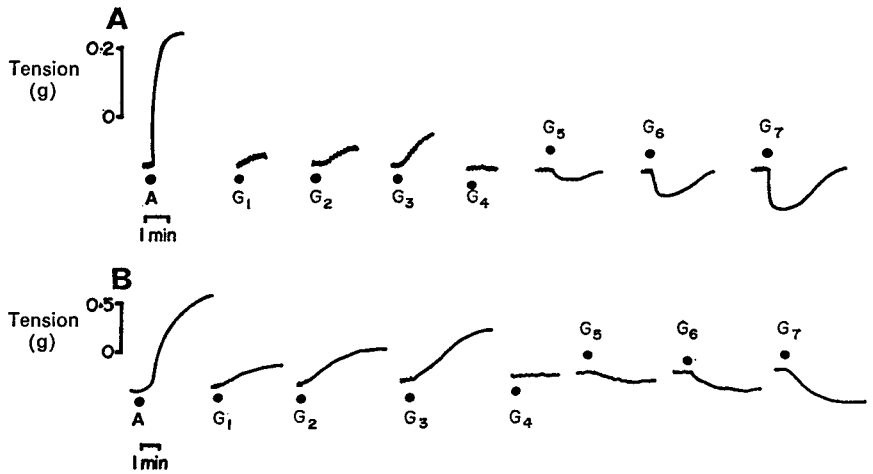

FIG. 1. Smooth muscle responses to acetylcholine and dibutyryl 3′,5′ GMP. (A) Guinea-pig ileum. Acetylcholine, A, 1 μg ml^{-1}. Dibutyryl 3′,5′ GMP: G_1, 7·5, G_2, 9·5, G_3, 11·5, G_4, 30, G_5, 75, G_6, 85, G_7, 100 μg ml^{-1}.

(B) Guinea-pig trachea. Acetylcholine, A, 0·5 μg ml^{-1}. Dibutyryl 3′,5′ GMP, G_1, 10, G_2, 12, G_3, 14, G_4, 30, G_5, 115, G_6, 120, G_7, 125 μg ml^{-1}.

may be a "second messenger" for cholinergic responses. Acetylcholine responses of both tissues consist of large, rapid contractions in comparison with the small, slowly developing contractions with the GMP nucleotides. Our results suggest that the contractile effect of 3′,5′ GMP and its analogue may be mediated by muscarinic receptors since atropine reduces the response. The effectiveness of atropine in blocking the contraction and of eserine in enhancing it also indicates that low doses of 3′,5′ GMP and dibutyryl 3′,5′ GMP probably contract smooth muscle by liberation of acetylcholine. The failure of dibutyryl 3′,5′ GMP to potentiate acetylcholine might be explainable in this context. These findings confirm those of Puglisi, Berti & Paoletti (1971) who showed that atropine abolished 3′,5′ GMP-mediated contractions in rat stomach fundus strip. The smaller, slower contractions of the nucleotides may be the result of permeability delays and a gradual release of acetylcholine stores from both tissues. Whether this release is from acetylcholine stores at nerve endings or is the result of ganglionic stimulation remains to be determined.

Relaxation by catecholamines of both ileum (Bueding, Butcher & others, 1966) and trachea (Murad, 1972) of the guinea-pig is accompanied by increased 3′,5′ AMP content. The release of tissue catecholamine stores by 3′,5′ GMP is not the cause for relaxation since its relaxant effects were not prevented by β-adrenoceptor blockade with propranolol. Schultz, Hardman & others (1972) have indicated that changes in the levels of 3′,5′ GMP and 3′,5′ AMP can occur independently of each other. It is possible that cyclic GMP nucleotides in high concentrations are converted to the 5′ GMP which relaxes smooth muscle. Alternatively, the cyclic GMP nucleotides may inhibit phosphodiesterase activity (Szaduykis-Szadurski, Weiman & Berti, 1972) and consequently increase cellular 3′,5′ AMP levels which would also produce smooth muscle relaxation.

Both 3′,5′ AMP and 3′,5′ GMP levels may be determinants of the contractile state of ileal and tracheal smooth muscle of the guinea-pig. The biphasic effect of 3′,5′ GMP emphasizes that the effects of these "second messengers", administered exogenously, are highly dependent upon dose level and that care should be taken in finally assessing the potency of major breakdown products in such studies. It is obvious that the mechanisms for the 3′,5′ GMP responses require further studies including

careful examination of endogenous 3',5' GMP changes during the smooth muscle responses. This work was supported by US PHS Grants HE14534 and HE14179. We thank Dr. J. F. Kuo for his interest and helpful discussion about this work.

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Similarity between the effects of dimethyl and monomethyl tricyclic drugs on reserpine effects in the frog and 5-hydroxytryptamine uptake by human blood platelets

We have previously observed that the tertiary (dimethyl) tricyclic antidepressants, imipramine and amitriptyline, are much stronger than their secondary (monomethyl) derivatives, desipramine and nortriptyline, in enhancing the effects of reserpine in the frog (Oxenkrug & Lapin, 1971). Our data were confirmed by Frank (1971). The effects of reserpine (loss of the righting reflex and appearance of twitches of the extremities) are presumably related to activation of the central 5-hydroxytryptamine (5-HT) processes (Lapin, Oxenkrug & others, 1970). The tertiary compounds are also reported to be more potent than secondary ones in blocking the uptake of 5-HT into central neurons (Carlsson, 1970) and into blood platelets (Todrick & Tait, 1969). The latter were a useful model for the neuronal uptake of 5-HT (see Pletscher, 1968).

Recently we compared the influence of aminopropyl derivatives (imipramine and desipramine) with that of the corresponding β-aminopropionyl derivatives (IPK-17 and IPK-18) of dibenzazepine on the effects of resperine in the frog and on the uptake of 5-HT by human blood platelets. Dimethyl (IPK-17) and monomethyl (IPK-18) β-aminopropionyl dibenzazepine (synthesized in Leningrad Technological Institute) have the imipramine-like pharmacological spectrum (Lapin, 1966; Lapin & Schelkunov, 1968).

Male frogs (*Rana temporaria*) were used as described earlier (Oxenkrug & Lapin, 1971). The uptake of 5-HT by human blood platelets was studied under conditions similar to those described by Ahtee & Saarnivaara (1971) with the exception that we precipitated proteins with 0·1N $HClO_4$. The statistical significance of the results was determined by Student's *t*-test.