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Non-competitive spasmolytics as antagonists of Ca^{++}-induced smooth muscle contraction

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The spasmolytic drugs papaverine, phenpropamine, Cxn 2 (1-methylamino-3,3,5-trimethylcyclohexane), khellin, cyclandelate, magnesium chloride, cocaine, 2,4-dinitrophenol and KCN antagonize various types of spasmogens, such as acetylcholine or histamine, non-competitively. They are assumed to act at some level in the excitation-contraction process common to the various spasmogens. Ca^{++} is essential for the induction of a contraction by the spasmogens. In the non-competitive action of some spasmolytics, Ca^{++} is postulated to be involved. To detect possible differences in their mechanism of action the various spasmolytics were tested against Ca^{++} acting as a pseudo-spasmogen on the K^{+}-primed taenia caeci of the guinea-pig in a Ca^{++}-free medium. Although different mechanisms of action for the various spasmolytics are to be expected, they all behaved similarly with respect to Ca^{++} in that they all caused a parallel shift in the log dose-response curve of Ca^{++} to higher concentrations.

Spasmolytics antagonize the contractions induced by spasmogens in smooth muscle tissue. The action of spasmogens involves two main sequences of biochemical events: the excitation in the muscle cell membrane and the contraction related to chemomechanical processes in this cell. In the coupling between excitation and contractile processes, Ca^{++} appears to play an essential role (Durbin & Jenkinson, 1961; Yukisada & Ebashi, 1961; Hurwitz, Battle & Weiss, 1962; Daniel, 1964; Woodbury, Gordon & Conrad, 1965; Bianchi, 1968; Oehme, Bergman & others, 1969; Ebashi, 1970).

Non-competitive spasmolytics (also called non-specific spasmolytics, musculotropic spasmolytics or antispasmodics) do not interact with the receptors of the spasmogens as do the competitive spasmolytics but interfere with the chain of events leading from the receptor occupation by the spasmogen to its effect. The fact that they are called musculotropic spasmolytics indicates that they do not interfere with the neuromuscular transmission but with the contraction process in the muscle fibres. The effect of this type of spasmolytic is insurmountable. This means that in their presence dose-effect curves for spasmogens on smooth muscle tissues are depressed. Papaverine is the prototype of this group of compounds; Mg^{++}, cocaine, cyclandelate, khellin, phenpropamine, Cxn 2 (1-methylamino-3,3,5-trimethylcyclohexane), KCN and 2,4-dinitrophenol have also been found to act as non-competitive spasmolytics (Ariëns, 1970). It is postulated that these non-specific spasmolytics may interfere at any level in the sequence of processes common to all spasmogens, that is, with the excitation-contraction coupling process or with the contraction process. However, the various members of the group may well act at different levels in this sequence. This is likely since the chemical characteristics of the compounds in question are very different.

The action of papaverine does not depend on the type of spasmogen—histamine,

acetylcholine, barium chloride, K^+—involved (Ariëns, 1970). Papaverine has nearly the same activity against the various spasmogens (Ariëns, 1970) but opinions differ on its mechanism of action. Santi, Contessa & Ferrari (1963) postulated an inhibitory action on oxidative phosphorylation, Holtz, Langeneckert & Palm (1968) a β-adrenergic mechanism of action, Kukovetz, Juan & Pöch (1969) suggested an inhibitory action on the phosphodiesterase while Ferrari & Carpenedo (1968a and b) proposed a competitive antagonistic action with respect to Ca^{++}. Interference with Ca^{++} was also assumed to play a role in the spasmolytic action of cocaine (Bianchi, 1968; Hurwitz, Battle & Weiss, 1962; Feinstein & Paimre, 1969) and Mg^{++} (Edman & Schild, 1962). It may be asked whether such an interference is restricted to these compounds and if on this basis a differentiation of the various non-competitive spasmolytics is possible.

In a Ca^{++}-free medium smooth muscle spasmogens fail to cause a contraction. Addition of Ca^{++} after a spasmogen results in a contraction. On this basis cumulative dose-response curves can be obtained for the contraction of smooth muscle by increasing the Ca^{++} concentration. Ca^{++} then acts as a pseudo-spasmogen. Some spasmolytics have now been tested on such a system as possible antagonists of Ca^{++}.

METHODS

Isolated taenia caeci of young guinea-pigs were used and maintained at 37° in Tyrode through which air was bubbled. On this organ primed with K^+ various spasmolytics were tested against Ca^{++}. Cumulative dose-response curves (van Rossum & van den Brink, 1963; van Rossum, 1963) for Ca^{++} were studied in the absence and in the presence of various concentrations of a spasmolytic. In the period between the experiments the organ was kept in Ca^{++}-free Tyrode solution. Three min before starting a cumulative dose-response curve with Ca^{++}, this solution was replaced by K_2SO_4-Ringer (21·6 g K_2SO_4, 0·2 g $NaHCO_3$ and 1 g glucose to 1 litre of distilled water) and at the start of each curve it was exchanged for KNO_3-Ringer (17·6 g KNO_3, 0·2 g $NaHCO_3$ and 1 g glucose to 1 litre of distilled water). According to Ferrari & Carpenedo (1968a) substitution of the sulphate-Ringer by the nitrate-Ringer has the advantage that precipitation of Ca^{++} is avoided while the contractions are well maintained. The intermediate use of the SO_4^{--}-medium enhances the elimination of Ca^{++}.

RESULTS

In the presence of $MgCl_2$ the log dose-response curves obtained with Ca^{++} on the K^+-primed taenia caeci showed a parallel shift to higher concentrations (Fig. 1A). This suggests a competitive relation between Ca^{++} and Mg^{++} as also reported by Edman & Schild (1962). Similar results were obtained with cocaine as an antagonist (Fig. 1B), which confirms competition with Ca^{++} as mentioned by Hurwitz, Battle & Weiss (1962) and by Feinstein & Paimre (1969). The parallel shift of the Ca^{++}-curve by papaverine as reported by Ferrari & Carpenedo (1968a,b) was confirmed, even over a large dose range (Fig. 1C). Other papaverine-like amines such as phenpropamine and Cxn 2 behaved in the same way. The question arises whether a correlation exists for these papaverine-related amines between their Ca^{++}-antagonizing potency and their non-competitive spasmolytic potency against spasmogens such as cholinergics and histamine in the classical test on the guinea-pig isolated ileum. Such a correlation was indeed found. The relative antagonistic potencies against both Ca^{++} and the

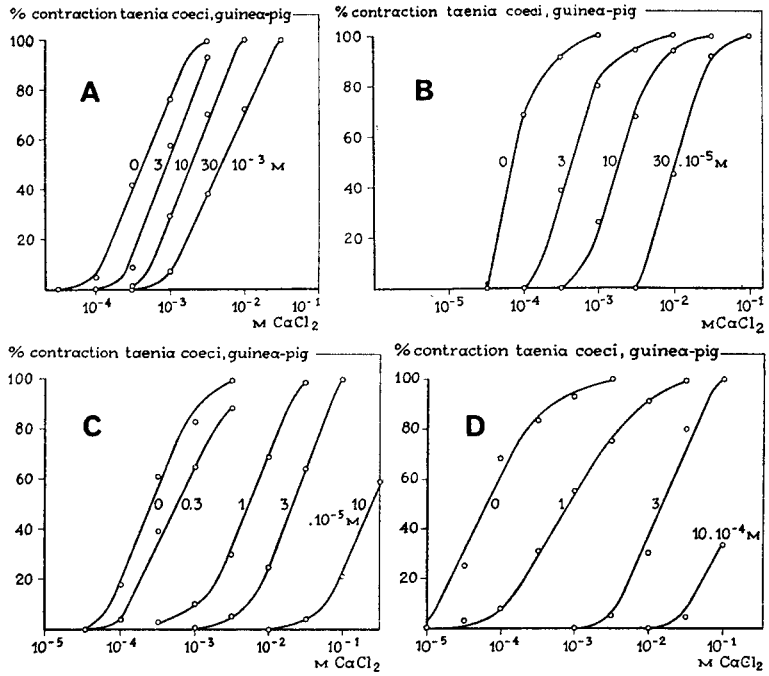

FIG. 1. Cumulative log dose-response curves for $CaCl_2$ as a pseudo-spasmogen on the K^+-primed isolated taenia caeci muscle of the guinea-pig in a Ca^{++}-free KNO_3-Ringer and the influence thereon of various non-competitive spasmolytics: (A) $MgCl_2$, (B) cocaine, (C) papaverine, (D) khellin. Note that all spasmolytics cause a parallel shift of the $CaCl_2$-curve to higher Ca^{++}-concentrations.

classical spasmogens for papaverine, phenpropamine and Cxn 2 were about 100, 1000 and 5 respectively. This indicates that the parallel shift observed with respect to Ca^{++} is probably related to the non-competitive papaverine-like activity of these drugs. Structurally unrelated non-competitive "papaverine-like" spasmolytics such as the non-ionizable nitrogen-free spasmolytics khellin (Fig. 1D) and the ester-type cyclandelate also displace the log dose-response curve obtained with Ca^{++} to the right of the control and in a parallel manner.

Remarkably, however, a metabolic blocker such as 2,4-dinitrophenol—a compound which acts as an uncoupling agent—also causes a parallel shift in the log dose-response curve obtained with Ca^{++}. Ferrari & Carpenedo (1968a) reported a similar phenomenon for KCN, also using the K^+-primed depolarized taenia caeci smooth muscle of the guinea-pig.

The non-competitive spasmolytics cyclandelate, papaverine, phenpropamine, Cxn 2, magnesium chloride, cocaine, khellin, 2,4-dinitrophenol and KCN all show a similar behaviour in their antagonism of Ca^{++} acting as a pseudo-spasmogen on the potassium-primed taenia caeci muscle of the guinea-pig, despite the fact that these spasmolytics very probably have different mechanisms of action. The desired differentiation of the various non-competitive spasmolytics was not obtained. The undifferentiated behaviour of the chemically heterogeneous and biochemically probably differently acting non-competitive spasmolytics in their action against Ca^{++} awaits further elucidation.

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REFERENCES

ARIËNS, E. J. (1970). In: *Proc. IV Int. Congress Pharmac.*, Basel, 1969, III, 16. Basel: Schwabe.

BIANCHI, C. P. (1968). *Cell calcium.* In: *Molecular biology and medicine series.* Editor: Bittar, E. E. London: Butterworth.

DANIËL, E. E. (1964). *Ann. Rev. Pharmac.*, **4**, 189–222.

DURBIN, R. P. & JENKINSON, D. H. (1961). *J. Physiol., Lond.*, **157**, 90–96.

EBASHI, S. (1970). *Proc. IV Int. Congress Pharmac.*, Basel, 1969, I, 32. Basel: Schwabe.

EDMAN, K. A. P. & SCHILD, H. O. (1962). *J. Physiol., Lond.*, **161**, 424–441.

FEINSTEIN, M. B. & PAIMRE, M. (1969). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **28**, 1643–1648.

FERRARI, M. & CARPENEDO, F. (1968a). *J. Pharm. Pharmac.*, **20**, 317–318.

FERRARI, M. & CARPENEDO, F. (1968b). *Archs int. pharmacodyn. Thér.*, **174**, 223–232.

HOLTZ, P., LANGENECKERT, W. & PALM, D. (1968). *Arch. exp. Path. Pharmak.*, **259**, 290–306.

HURWITZ, L., BATTLE, F. & WEISS, G. B. (1962). *J. gen. Physiol.*, **46**, 315–332.

KUKOVETZ, W. R., JUAN, H. & PÖCH, G. (1969). *Arch. exp. Path. Pharmak.*, **264**, 262.

OEHME, P., BERGMANN, J., RUDEL, M., EICHSTADT, M. & JUNG, F. (1969). *Archs int. Pharmacodyn. Thér.*, **179**, 6–22.

ROSSUM, J. M. VAN (1963). *Ibid.*, **143**, 299–330.

ROSSUM, J. M. VAN & VAN DEN BRINK, F. G. (1963). *Ibid.*, **143**, 240–246.

SANTI, R., CONTESSA, A. R. & FERRARI, M. (1963). *Biochem. Biophys. Res. Commun.*, **11**, 156–159.

WOODBURY, J. W., GORDON, A. M. & CONRAD, J. T. (1965). *Muscle*, Ch. 5. In: *Physiology and Biophysics.* Editors: Ruch, T. C. & Patton, H. D. Philadelphia: Saunders.

YUKISADA, N. & EBASHI, F. (1961). *Jap. J. Pharmac.*, **11**, 46–53.