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SHORT COMMUNICATIONS

Comparative actions of the trimetoquinol, tetrahydropapaveroline and salsolinol isomers in β-adrenoceptor systems

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We reported earlier [1, 2] on the stereoselective interaction of trimetoquinol and related tetrahydroisoquinolines (THI) in the α- and β-adrenoceptor systems, guinea pig aorta and rat adipose tissue respectively. The interaction of 1-substituted THI's in lipolysis was best accommodated by isomers possessing an S absolute configuration and a large group at the asymmetric 1-carbon position of the THI nucleus (see structures in Fig. 1). On guinea pig aorta, the isomers were observed to be equieffective competitive α-adrenoceptor blockers.

The THI's, salsolinol and tetrahydropapaveroline, have received considerable attention due to their formation from the biogenic amines, *in vivo* and *in vitro*, and proposed involvement in alcoholism and Parkinson's disease during treatment with L-dopa [3, 4]. Until recently [2, 5], little evidence was available concerning the β-adrenoceptor activity of the stereoisomers of these THI's. In an attempt to explore the differential actions of the THI's, we have examined the dose–response effects of trimetoquinol, tetrahydropapaveroline and salsolinol isomers in guinea pig tracheal and atrial preparations. These findings may provide leads to the development of a selective bronchial relaxant which would be beneficial for the treatment of respiratory diseases in patients with cardiovascular problems.

For the isolated guinea pig tracheal strip and right atrial preparations, non-fasted albino guinea pigs of either sex weighing 300–500 g were used. The isolation and mounting of tissues in a 12-ml jacketed muscle chamber containing physiological solution maintained at 37° and bubbling with a gas mixture (95% O_2:5% CO_2) were carried out as described previously [6, 7]. Our experiments did not include the use of an α-adrenoceptor blocker or catechol-*O*-methyltransferase inhibitor. Tissues were allowed to equilibrate for 1 to 1·5 hr. Carbachol (3×10^{-7} M) was used to increase the tone of the tracheal preparations. Drug-induced effects were recorded on a Grass polygraph (model 7C) via force displacement transducer, and cumulative dose–response curves were obtained for each isomer. Drug responses are expressed in terms of the maximum response obtained in the presence of (−)-isoproterenol (10^{-5} M) added at the end of each experiment. All compounds were prepared in saline containing 0·05% metabisulfite.

The comparative tracheal relaxant actions of the isomers of trimetoquinol, tetrahydropapaveroline and salsolinol are presented in Fig. 1. The chemical structure and position of asymmetry in each THI are also shown. In this preparation, S-(−)-trimetoquinol and S-(−)-tetrahydropapaveroline were observed to be more active than their corresponding R-(+)-isomers. The isomeric activity differences (negative log ED_{50} for S-(−)-isomer minus negative log ED_{50} for R-(+)-isomer) for trimetoquinol and tetrahydropapaveroline were calculated to be 2·0 and 0·5 log units respectively. The small difference between the isomers of tetrahydropapaveroline in trachea is similar to the results observed in rat adipose tissue [2]. By contrast, the isomers of salsolinol were found to be nearly devoid of tracheal relaxant properties. Moreover, it should also be noted that none of the THI's tested were able to produce a relaxation of trachea equal to the maximal effect obtained with 10^{-5} M (−)-isoproterenol.

The influence of these THI's on the chronotropic response of guinea pig atria is given in Fig. 2. All compounds possessed stimulatory activity. As noted in guinea pig trachea, the S-(−)-isomers of trimetoquinol and tetrahydropapaveroline were more active than the R-(+)-isomers. Even though the salsolinol isomers showed a weak stimulatory effect, no significant difference could be detected between the optical isomers. It is clear that the isomeric activity differences (IAD) obtained for tetrahydropapaveroline in atria (1·3 log units) were greater than in trachea whereas the IAD's for trimetoquinol were nearly equival-

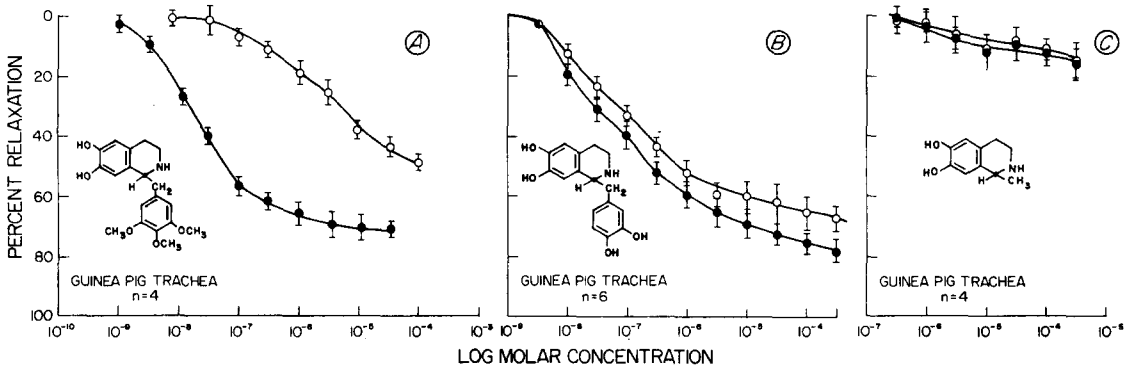

Fig. 1. Log dose–response curves for the isomers of trimetoquinol, tetrahydropapaveroline and salsolinol on the relaxation of guinea pig tracheal strips. Key: (A) S-(−)-trimetoquinol (●——●) and R-(+)-trimetoquinol (○——○); (B) S-(−)-tetrahydropapaveroline (●——●) and R-(+)-tetrahydropapaveroline (○——○); and (C) S-(−)-salsolinol (●——●) and R-(+)-salsolinol (○——○). Chemical structures of each THI derivative are presented in each frame together with the center of asymmetry denoted by the ● within each structure. Values plotted represent the mean ± S. E. of N = 4–6.

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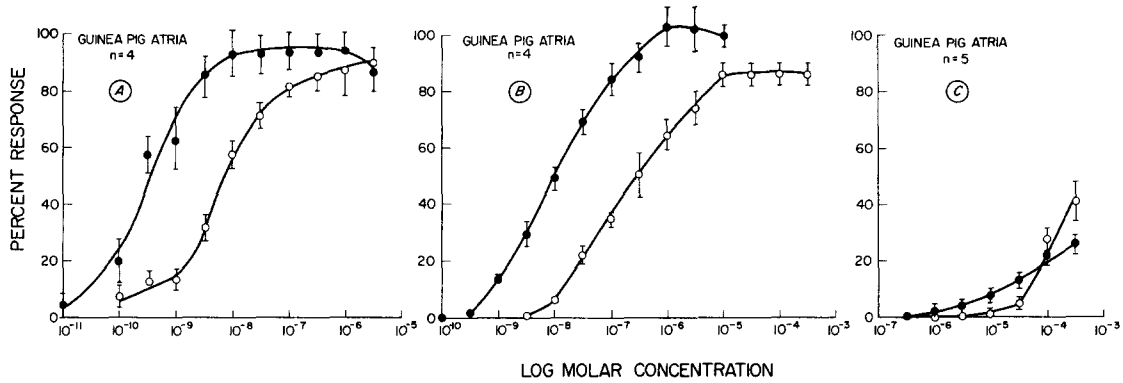

Fig. 2. Log dose–response curves for the isomers of trimetoquinol, tetrahydropapaveroline and salsolinol on the heart rate of isolated spontaneously beating right guinea pig atria. Key: (A) S-(−)-trimetoquinol (●——●) and R-(+)-trimetoquinol (○——○); (B) S-(−)-tetrahydropapaveroline (●——●) and R-(+)-tetrahydropapaveroline (○——○); and (C) S-(−)-salsolinol (●——●) and R-(+)-salsolinol (○——○). Values plotted represent the mean ± S. E. of N = 4–5.

ent in both β-adrenoceptor systems. These findings with trimetoquinol in both β-adrenoceptor systems are similar to the recent report of Buckner and Abel[8].

Lands *et al.* [9] have subclassified β-adrenoceptors in heart muscle and bronchial muscle as β-1 and β-2 respectively. In this regard, our data coupled with the work of Buckner and Abel [8] demonstrate that trimetoquinol is a potent agent in β-adrenoceptor systems of both β-1 (heart muscle, lipolysis) and β-2 (bronchial muscle). These findings differ considerably from earlier reports [10, 11] on the chronotropic and ionotropic action of trimetoquinol in guinea pig heart. Farmer *et al.* [10] and Brittain *et al.* [11] provided evidence that the racemate of trimetoquinol is a weak stimulant of guinea pig heart and is, therefore, a selective stimulant of β-2 adrenoceptors. Although not presented, we found that racemic trimetoquinol is approximately one-half as active as the S-(−)-isomer in the stimulation of chronotropic response in guinea pig atria. Thus, the use of the racemate or stereoisomers of trimetoquinol does not account for the discrepancies noted in the various laboratories. Buckner and Abel[8] have observed that the time–response relationship for trimetoquinol is considerably longer than with isoproterenol in β-adrenoceptor systems and further suggested that equilibrium conditions may not have been achieved in the earlier studies[10, 11]. This observation may serve to adequately explain the divergent potency differences reported for trimetoquinol in guinea pig heart preparations. A resolution of these differences is an important factor which should be considered in the evaluation and development of selective β-stimulants.

The demonstration of β-adrenoceptor activity for THI alkaloids preceding this study has involved the use of racemic mixtures [10, 13]. The present report verifies the importance of asymmetry and of the substituent present at the 1-carbon position of the THI nucleus. As presented, β-stimulant properties of THI's reside primarily with isomers of the S absolute configuration. The stereoselective interaction of THI's confirms the recent report of Buckner and Abel [8] and is in agreement with earlier studies in lipolysis [1, 2, 5] and trachea [14]. It is also clear that the presence of a large substituent at the 1-position is concomitant with potent β-adrenoceptor activity for this chemical class of compounds. In both β-adrenoceptor systems, the relationship between biological activity and nature of the 1-substituent on the THI nucleus was trimethoxybenzyl > dihydroxybenzyl ≫ methyl (see Fig. 1). We found an identical relationship for the interaction of these THI's in rat adipose tissue [2].

Hypotheses proposed to explain the pharmacological and toxicological properties of THI alkaloids formed *in vivo* have been associated with their ability to (a) act as potent β-stimulants and/or (b) interfere with adrenergic neurotransmission (e.g. false neurotransmitter) [5, 11, 12, 15]. Based upon our findings, it has been shown that salsolinol is a weak β-adrenergic stimulant and it seems unlikely that the effects associated with formation of this THI are due to a direct activation of adrenoceptors, *in vivo*. On the other hand, tetrahydropapaveroline is a relatively potent β-stimulant and a direct stimulation of adrenoceptors may contribute significantly to the action *in vivo* of this compound. Nevertheless, it is evident that additional studies are necessary to fully clarify the over-all mechanism(s) of sympathomimetic action of THI alkaloids.

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Vitamin B_{12} levels in drug-treated bacterial cells

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Up till now little attention has been paid to the action of bacteriostatics on the biosynthesis of cobalamins. In the presence of sulfathiazole and aureomycin, the content of a biologically active form of vitamin B_{12} synthesized by some species of Propionibacteria was found to be increased. However, this phenomenon has so far not been theoretically explained [1, 2]. On the other hand, Bukin and Proniakova found that sulfathiazole in the concentration of 1 mg% caused a marked inhibition of vitamin B_{12} formation by *Propionibacterium shermanii*, without influencing the yield of the bacterial mass [3]. According to Cohen the synthesis of vitamins was generally inhibited by sulfathiazole [4]. Kanopkajtie-Rozgiene found that an increase in vitamin B_{12} content in the biomass of *Azotobacter chroococcum* occurred only at the lag phase of growth [5]. In the previous studies an increase in the level of vitamin B_{12} was found in cells treated with 1·2% sulfathiazole, as compared with the B_{12} level in control cells of the mesophilic strain of *Bacillus megatherium* in their logarithmic phase of growth [6]. This fact has led the author to examine whether this phenomenon was connected with a specific action of this drug on vitamin B_{12} synthesis, or if it was due to changes in the metabolic pattern of the drug-treated cells. In these studies, for comparison, amethopterin, 5-fluorouracil, chloramphenicol, actinomycin D and puromycin were used. The experiments were performed on the mesophilic strain of *B. mehatherium*.

Culture conditions. Bacillus megatherium was grown in the following fermentation medium: broth 20 g, molasses 10 g, L-methionine 40 mg, Co (as chloride) 5 mg and water 1 litre. Prior to inoculation, the drugs were introduced into the culture media and afterwards the cells were seeded. The optical density of the cultures was then adjusted to about 0·3. The media in 60-ml amounts were distributed into 100-ml flasks. The cultures were kept at 30° in a shaker. At the experimental time periods the cells of control cultures and those of drug-treated cultures were centrifuged at 9000 rev/min. In parallel test samples the content of vitamin B_{12} per 1 mg of protein and the optical density of the cultures were estimated. Extraction of vitamin B_{12} from bacterial cells was performed by the method of Pawełkiewicz and Zodrow [7]. Vitamin B_{12} was determined microbiologically with *Lactobacillus leichmanii* ATCC 7830. Nephelometric readings were taken on a Coleman spectrophotometer, Model 14 at 600 nm. To determine the protein content in the test samples the method of Folin-Ciocalteau was employed [8].

Drugs. Sulphathiazolum solubile FP IV, sal. natric., Starogardzkie Zakłady Farmaceutyczne-Polfa was used at 0·05%, 5-fluorouracil sub forma sal. nitric., La Roche at

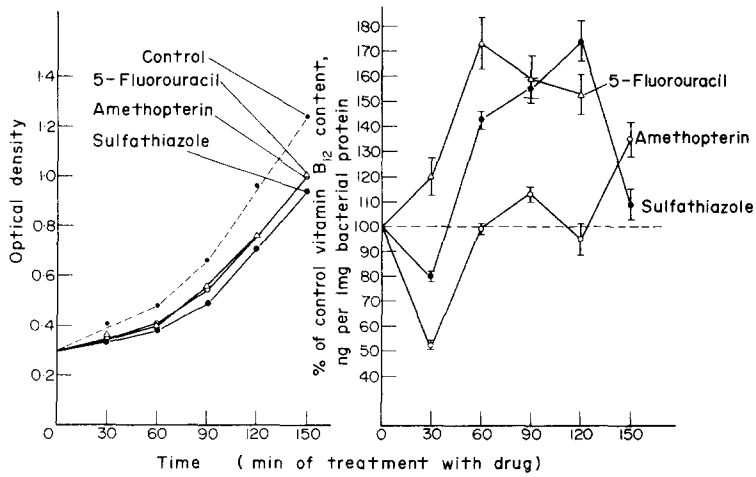

Fig. 1. Effects of sulfathiazole, amethopterin and 5-fluorouracil on the growth of cultures and on vitamin B_{12} content in cells of *B. megatherium*. Cell growth with sulfathiazole at the concentration of 0·05%, with 10^{-4} M amethopterin and with 0·15 mg/ml 5-fluorouracil. Points at 30 min on the B_{12} curve for sulfathiazole and amethopterin-treated cells differ significantly from corresponding point of control value ($P = 0·001$). Points at 30, 60, 90 and 120 min on the B_{12} curve for 5-fluorouracil-treated cells differ significantly from corresponding points of control values ($P = 0·05$ or $P = 0·01$ or $P = 0·001$).