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SHORT COMMUNICATIONS

Trimethoquinol—different pharmacological properties of optical isomers

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It is usual to find that one of the optical isomers of a pharmacologically active agent is more active than its enantiomer. Trimethoquinol, 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (TMQ), is just such an example. For pharmacological activity in the lung [1–3], heart [2–4] or adipose tissue [5], there is definite stereochemical selectivity for S-(−)-1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline. In various preparations *in vitro*, S-(−)-TMQ was about twice as active as the racemate [1, 6, 7]. R-(+)-TMQ, on the other hand, had weak cardiovascular and bronchodilator activity [1, 7] and R-(+)-TMQ did not antagonize the bronchodilator activity of the S-(−)-isomer [1, 7].

Recently, we found racemic TMQ to have potent platelet anti-aggregatory properties in human platelet-rich plasma and washed human platelets [8]. The mechanism of action of TMQ for inhibition of platelet aggregation appeared to be different from that for bronchodilation, myocardial stimulation and mobilization of free fatty acids [8]. In order to explore this possible difference in mechanism of action of TMQ in different tissues, we have studied TMQ isomers for their effects on both platelets and bronchial smooth muscle and have found opposite stereoselective requirements in these two tissues. Inhibition of platelet aggregation, *in vitro*, is selective for R-(+)-1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, whereas bronchodilatory activity is selective for the S-(−)-isomer.

For platelet aggregation studies, venous blood was obtained from human volunteers who had not taken aspirin for at least 7 days. It was collected in siliconized 20 ml Vacutainer tubes (Becton Dickinson & Co., Rutherford, N.J.) fitted with 20-gauge needles using 3.8% sodium citrate as the anticoagulant (9 vol blood to 1 vol sodium citrate). Platelet-rich plasma (PRP) was separated from the red blood cells by centrifugation for 15 min at 180 *g* at room temperature. Platelet-poor plasma (PPP) was prepared by centrifuging PRP for 2 min at 1000 *g*. Techniques established by Born and Cross [9] were used to study platelet aggregation *in vitro* employing a Dual Channel Aggregation Module (Payton Associates, Inc., Buffalo, N.Y.). One ml PRP was added to a siliconized cuvette containing a siliconized stirring bar, placed in a densitometer maintained at 37° and stirred at 1000 rev/min. Various concentrations of test compounds dissolved in 50 μl saline were added and preincubated with PRP for 5 min. Aggregation was initiated by the addition of ADP (2.5 μM), epinephrine HCl (45 μM) or human mammary gland collagen (kindly donated by Dr. Harvey Weiss, Roosevelt Hospital, N.Y.) sufficient to give about 60 per cent of the maximum aggregation response, as defined by transmission through PPP.

Bronchodilation was studied in male guinea pigs (350–500 g) which were anesthetized with urethane (2.0 g/kg, i.p.) and given gallamine triethiodide (3 mg/kg, i.p.) as a muscle relaxant. The trachea was cannulated and the lungs were inflated with air by a Phipps & Bird small animal respirator, maintained at 45 strokes/min. Stroke volume was adjusted to provide an air overflow at a constant pressure of 8–10 cm H_2O. Ventilation pressure was recorded after various i.v. doses of histamine from which a "pre-drug ventilation pressure" and a control histamine dose were selected for each animal. After i.v. injection of TMQ, histamine was introduced in increasing doses until the selected pre-drug ventilation pressure was achieved. The ratio of the histamine dose needed after TMQ administration/control histamine dose was calculated and plotted as a function of TMQ dose. When a drug caused a decrease in airway resistance, a greater amount of histamine was needed to produce the pre-drug ventilation pressure and this resulted in an increase in the ratio of histamine doses. Therefore, ratios > 1.0 represented a decreased airway resistance and reflect bronchodilator activity and values < 1.0 indicated an increased airway resistance resulting from bronchoconstrictor activity [10].

TMQ inhibited collagen, ADP and epinephrine-induced platelet aggregation in human citrated platelet-rich plasma (Fig. 1). A high degree of stereoselectivity was evident in the inhibitory activity of TMQ on platelet aggregation. The R-(+)-isomer was significantly more potent than the S-(−)-isomer and the R-(+)-/S-(−) ratios of inhibitory

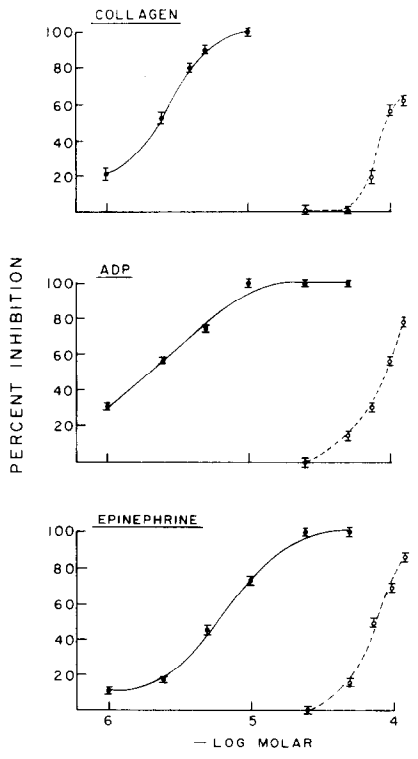

Fig. 1. Comparative activity of the trimethoquinol optical isomers as inhibitors of platelet aggregation in human citrated platelet-rich plasma. Key: R-(+)-trimethoquinol (●——●) and S-(−)-trimethoquinol (○——○). Concentrations of aggregating agents: 2.5 μM ADP, 45 μM epinephrine HCl. Values plotted represent the mean ± standard error of four or five determinations.

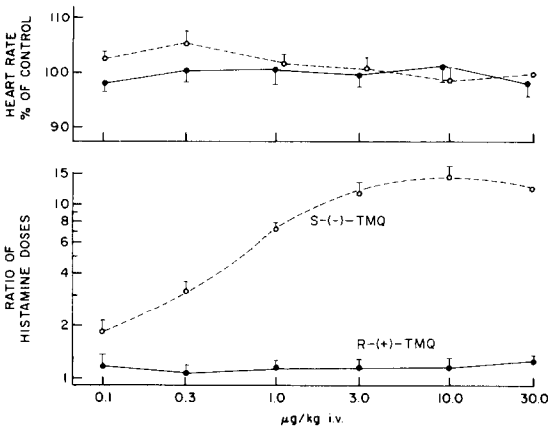

Fig. 2. Effect of optical isomers of trimethoquinol on heart rate and airway resistance in the anesthetized guinea pig. Key: R-(+)-trimethoquinol (●——●) and S-(−)-trimethoquinol (○——○). Each point represents the mean ± standard error for three or four animals.

activity were similar against each of the platelet aggregating agents studied (Fig. 1).

In contrast, the R-(+)-isomer of TMQ did not affect airways resistance in the anesthetized guinea pig while the S-(−)-TMQ caused a significant decrease (Fig. 2). The intravenous administration (0.1 to 30 μg/kg) of S-(−)-TMQ caused a decrease in airway resistance with maximum responses at 3–10 μg/kg, whereas comparative doses of R-(+)-TMQ had no effect. Neither optical isomer influenced heart rate in the guinea pig at the bronchodilatory doses studied.

TMQ has activity in many isolated smooth and cardiac muscle preparations [1, 3, 4], in spermatic duct [11] and in epididymal fat tissue [12] and has corresponding activities *in vivo* in many species [6, 7, 13] including man [14]. In all of these experiments, TMQ has a common mechanism of action involving the β-adrenergic receptor. Adrenoceptor agonist activity is characterized by the tissue response, specific blockade by β-adrenoceptor antagonists [1], activation of adenylate cyclase [15] and elevation of tissue cAMP levels [16]. Hence, the observed common stereoselective requirement for S-(−)-TMQ, in adrenergically responsive tissues, is an expected result. Inhibition of platelet aggregation, however, is not mediated by an adrenergic mechanism because inhibitory activity is not reversed by α- or β-adrenergic blockers and TMQ did not activate platelet adenylate cyclase or affect cAMP accumulation in human platelets [8]. In addition, the R-(+)-isomer of TMQ is more powerful as an inhibitor of platelet aggregation than S-(−)-TMQ. Thus, in the platelet, a different mechanism of action of TMQ is associated with a different stereoselectivity.

Stereochemical selectivity is a well recognized biological phenomenon. Once the active isomer of a drug molecule is identified as responsive in one tissue, it generally holds true for other biological preparations, and the active enantiomer of new related drugs can be predicted. Isomer activity has usually been thought to be independent of the pharmacological system studied. Our observations based on two different pharmacological preparations indicate a novel separation of pharmacological activities based on optical isomerism. Such a separation is not only of theoretical interest but could have considerable therapeutic value. Racemic TMQ inhibition of platelet aggregation was of limited clinical importance because the blood levels of TMQ required for platelet inhibitory activity would also cause bronchodilation and hypotension and would increase the heart rate. R-(+)-TMQ has negligible effects on the bronchial or heart muscle; in our experiments, therefore, it might be possible in man to achieve blood levels of R-(−)-TMQ sufficient to inhibit platelet aggregation without compromising other tissues. Conversely, adequate bronchodilator doses of S-(−)-TMQ in man might not change platelet function.

In connection with our observation of different pharmacological properties associated with different optical isomers, we note another example in which the biological activities of optical isomers of a drug have been reversed in a different test situation. (−)-Isoproterenol is consistently more active than (+)-isoproterenol when tested for heart rate, blood pressure and tracheal relaxation, but for lowering the intraocular pressure of the rabbit eye (+)-isoproterenol is more potent than the (−)-form [17].

Our experimental results suggest that an investigation of stereochemical selectivity of drugs in other tissues may be a useful approach to the development of organospecificity in drug therapy.

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REFERENCES

1. Y. Iwasawa and A. Kiyomoto, *Jap. J. Pharmac.* **17**, 143 (1967).
2. C. K. Buckner and P. Abel, *J. Pharmac. exp. Ther.* **189**, 616 (1974).
3. D. R. Feller, R. Venkatraman and D. D. Miller, *Biochem. Pharmac.* **24**, 1357 (1975).
4. H. Nakajima, M. Hoshiyama and A. Kiyomoto, *Nippon Yakurigaku Zasshi* **65**, 34 (1969).
5. R. F. Shonk, D. D. Miller and D. R. Feller, *Biochem. Pharmac.* **20**, 3404 (1971).
6. A. Kiyomoto, M. Sato, T. Nagao and H. Nakajima, *Eur. J. Pharmac.* **5**, 303 (1969).
7. A. Kiyomoto, Y. Iwasawa and S. Harigaya, *Arzneimittel-Forsch.* **20**, 46 (1970).
8. G. Shtacher, H. J. Crowley and C. Dalton, *Biochem. Pharmac.*, **25**, 1045 (1976).
9. G. V. R. Born and M. J. Cross, *J. Physiol., Lond.* **168**, 178 (1963).
10. H. Konzett and R. Rossler, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **195**, 71 (1940).
11. Y. Iwasawa and A. Kiyomoto, *Nippon Yakurigaku Zasshi* **63**, 28 (1967).
12. D. R. Feller, R. F. Shonk and D. D. Miller, *J. Pharm. Pharmac.* **22**, 869 (1970).
13. M. Sato, I. Yamaguchi and A. Kiyomoto, *Jap. J. Pharmac.* **17**, 153 (1967).
14. Y. Yamamura and S. Kishimoto, *Ann. Allergy* **26**, 504 (1968).
15. C. Grunfeld, A. P. Grollman and O. M. Rosen, *Molec. Pharmac.* **10**, 605 (1974).
16. M. Inamusu, A. Shinjo, Y. Iwasawa and T. Morita, *Biochem. Pharmac.* **23**, 3213 (1974).
17. R. J. Seidehamel, K. W. Duncan and T. E. Hickey, *Am. J. Opthal.* **79**, 1018 (1975).