HUMAN 5-HYDROXYTRYPTAMINE VENOMOTOR RECEPTORS

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SUMMARY The venoconstriction test (VCT) is a method for exploring the monoamine venomotor receptors in man: it is a painless, innocuous technique, able to give the maximum information with the minimum trouble, according to the requirements of the clinical pharmacological tests. The VCT records the local venospasm when very small amounts of 5-HT, noradrenaline or epinephrine are administered through a needle inserted orthodromically into the vein. The VCT is a suitable technique for an acceptable pharmacological evaluation of the vascular reactivity to 5-HT in man. The threshold effect, even if different from subject to subject, is quite constant in the same subject; the threshold doses of 5-HT in the majority of adult men and women, range between 100 and 500 ng. The inhibiting activity of anti-serotonin drugs such as LSD-25, BOL-148, methysergide, nicergoline, BC-105, cyproheptadine, and MY-25 is evaluated in man in quite a satisfying way. An unexpected result was the enhancement of 5-HT and noradrenaline reactivity by treatment with monoamine oxidase inhibitors. Supersensitivity to 5-HT but not to noradrenaline has been observed following administration of parachlorophenylalanine, a specific 5-HT depletor.

Skin reaction, capillary permeability, blood pressure and blood flow are used to test the vascular activity of 5-hydroxytryptamine (Fontanini et al. 1958; Scherbel and Harrison, 1958; Halpern et al. 1960): however, these parameters, when applied in human pharmacology, are quite unsuitable because of technical difficulties, scarce sensitivity and reproductivity.

Human veins are very sensitive to the spasmogenic action of 5-HT (Magalini et al. 1956). However the venous tone is quite difficult to measure because of the influence of the autonomous nervous system and of the humoral agents (Duggan et al. 1953; Sharpey-Schafer and Ginsburg, 1962; Sharpey-Schafer, 1963). A few years ago a technique was arranged to test the sensitivity of the 5-HT venomotor receptors in man (Sicuteri et al. 1964). This technique (venoconstriction test: VCT) is based upon the high responsiveness to 5-HT of the vein. The method is highly sensitive, being able to detect the activity of fractions of micrograms of 5-HT; it is practically painless and innocuous, in view of the exiguity of the doses; and the results are quite constant and reproductive. VCT has been used successfully to test agonists, antagonists and correlated drugs on the monoamine (5-HT and catecholamines) venomotor receptors: in the present paper we have collected the most significant information on the human pharmacology of the 5-HT venomotor reactivity, reported fragmentarily in short communications (Sicuteri et al. 1964a; 1965; 1966).

METHODS Volunteers: subjects were instructed about the method and the purpose of the experiments. Information concerning the drugs and their action were given: in particular the small amount of the doses was stressed, and the fact that only local effects were to be expected. Volunteers were physicians, technicians, patients of the Department of Clinical Pharmacology (non sufferers of infective haematological or cardiovascular disease), outpatients of the Headache Center, and the authors of the paper themselves.

One hundred and forty subjects (sixty men and eighty women), ranging in age from 20 to 55, were tested. The patients were given no drugs 20 days before the test. Chronic treatments with 5-HT antagonists, monoamine oxidase inhibitors, reserpine and parachlorophenylalanine were carried out on the patients who needed such therapy for headache and/or depression or arterial hypertension.

Instruments and procedure: An Ovlson needle (0.8 mm internal diameter) is connected through a tephlon tube with a syringe and a pressure transducer (type P23DB, Statham Laboratories Inc., Hato Rey Puerto Rico). (Fig. 1) The syringe pushed by an electric apparatus (Perfusor mod. IV, B.Braun, Melsungen, Germany) continuously perfuses saline (0.5 ml per min) into the tephlon tube. The pressure values are preamplified (Electromanometry Preamplifier, mod. KEMT.a, O.T.E. Montedel, Florence, Italy) and recorded (Multi Channel Recorders C2a O.T.E. Montedel, Florence, Italy).

APPARATUS FOR VENOCONSTRICTION TEST (V.C.T.)

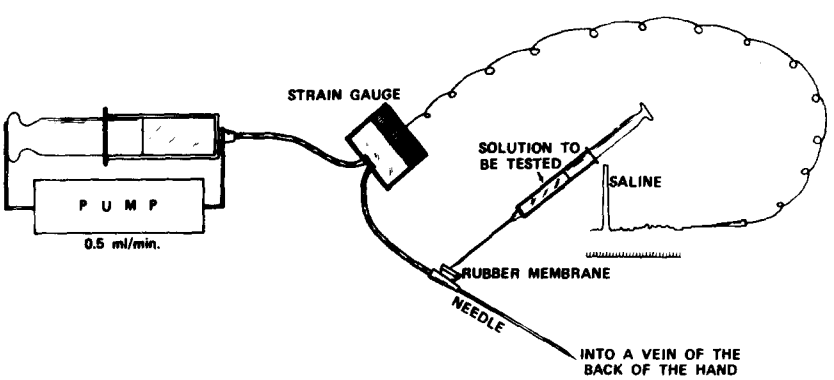

Fig. 1. The electric pump perfuses saline into the vein through the tephlon tube, the pressure Transducer and Ovlson needle. The drugs are inserted through the rubber membrane of the needle, and the pressure change is recorded.

The needle is inserted orthodromically, into a vein; generally a vein of the back of the hand or of the wrist is used. After a few minutes 0.5 ml of saline is administered through the rubber membrane of the needle, and such administration is repeated twice after a 3 minute interval.

Successively 5-HT and/or other drug solutions in 0.5 ml are inoculated at adequate intervals. Each drug administration is usually alternated with saline inoculation. VCT lasts for 1-3 hours and it can be repeated preferably after a few days.

Rationale: The fixed components of the VCT are: a) valves of the vein preventing the blood backflow; b) the speed of the infusion. The variable element is only the temporary reduction or obstruction of the vessel lumen a few centimeters around the needle, when a drug able to cause a spasm in the vein is injected. Thus, when saline is administered, no increase of resistance to the infusion will occur. If we insert a solution containing a sufficient amount of 5-HT or other prompt constrictor substances, the vein just around the point of the needle will show a spasm.

With this method a kind of small pressure chamber will be achieved, this is formed by the valves and by the vein walls (Fig.2). When the vein walls constrict, the resistance to the infusion will be enhanced. The occurrence and duration of the increased resistance to the infusion will depend upon the intensity and the duration of the venospasm.

The sensitivity to the 5-HT is usually evaluated according to the criterion of the threshold effect. The threshold increase of the infusion resistance is considered arbitrarily not inferior to 3 mmHg. For this we inject increasing doses of 5-HT, generally starting at 50 ng, until we reach the threshold effect (Fig. 3); 5-hydroxytryptamine creatinine sulphate was used and all values must be referred to this salt. When it was required the 5-HT sensitivity was compared with that of noradrenaline and adrenaline.

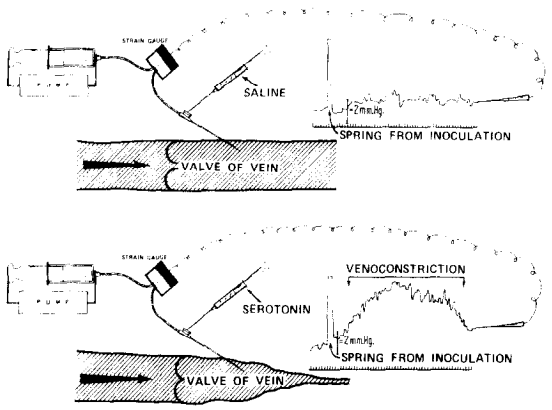

Fig. 2. While saline in the test vein does not provoke any increase of local venous pressure (above), 5-HT does. The local pressure increase is probably due to a sort of pressure chamber formed by valves and constricted vein walls.

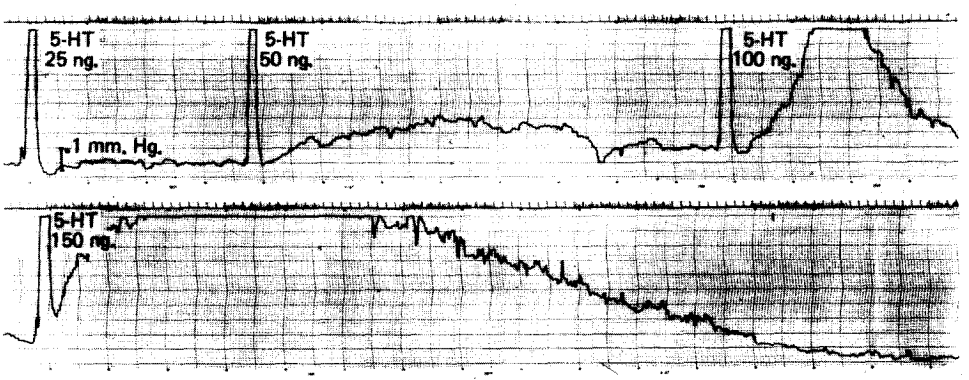

Fig. 3. Vein constriction induced by 5-HT: an example of dose-effect relationship.

Drugs: 5-hydroxytryptamine creatinine sulphate (2.5 mg/ml) and 1,6-dimethyl-8 beta-(5-bromonicotinoyl-oxymethyl)-10 alpha-methoxyergoline (nicergoline, Sermion: 1 mg/ml). Farmitalia, Milan, Italy.

Lysergic acid diethylamide (LSD-25:0.1 mg/ml);

1-methyl-d-lysergic acid butanolamide (Methysergide, UML-491, Deserril: 0.5 mg/ml);

2-bromo-d-lysergic acid diethylamide (BOL-148: 0.5 mg/ml);

1-methyl-cycloheptathiophene (BC-105, Sandomigran: 2mg/ml)and

1-methyl ergotamine tartrate (MY-25: 1 mg/ml): Sandoz, Basel, Switzerland.

Cyproheptadine (Periactin: 2 mg/ml): Merck, Sharp & Dohme, Rahway, N.J., U.S.A.

Phenelzine sulphate (Nardil: 15 mg/tablets): Angiolini, Milan, Italy; from Warner-Chilcott, Morris Plains, N.J., U.S.A.

Pargyline Hydrochloride (Eutonyl: 10 mg/tablets): Abbott Laboratories, North Chicago, Ill., U.S.A.

Nor-epinephrine bitartrate (Noradrec: 1 mg/ml) and

Epinephrine hydrochloride: (Adrenaline: 1 mg/ml): Recordati Laboratories, Milan, Italy.

p-Chlorophenylalanine (Fenclonine: 200 mg/capsules): Falorni, Florence, Italy.

Reserpine (Serpasil: 0.25 mg/tablets): Ciba, Basel, Switzerland.

Serum and plasma were obtained from the same subject tested with VCT.

Plasma poor in platelets was obtained by collecting blood in tephlon tubes with EDTA. Centrifugation was carried out at 300 r.p.m. for 30 minutes.

Post-ischemic plasma was obtained by drawing blood from the cubital vein 10 minutes after the interruption of blood flow by means of a sphygmomanometer cuff.

RESULTS

1) The sensitivity of the 5-HT venomotor receptors, evaluated by the threshold effect parameter, varies individually from a minimum of 50 ng to a maximum of 2 mcg. No difference

between male and female subjects was observed. The sensitivity, though varying from one subject to another, appears quite stable in the same person on different days. If threshold amounts of 5-HT are administered at intervals not being less than 5 minutes, tachyphylaxis does not occur.

2) By using the same criterion of the threshold effect, the ratio of activity between 5-HT and catecholamines is roughly the following: 5-HT; 2 NA; 4 A.

3) Human serum (2-4 ml) constricts the vein; this spasmogenic activity is due to 5-HT as the inhibition by antiserotonin drugs proves (Fig. 4).

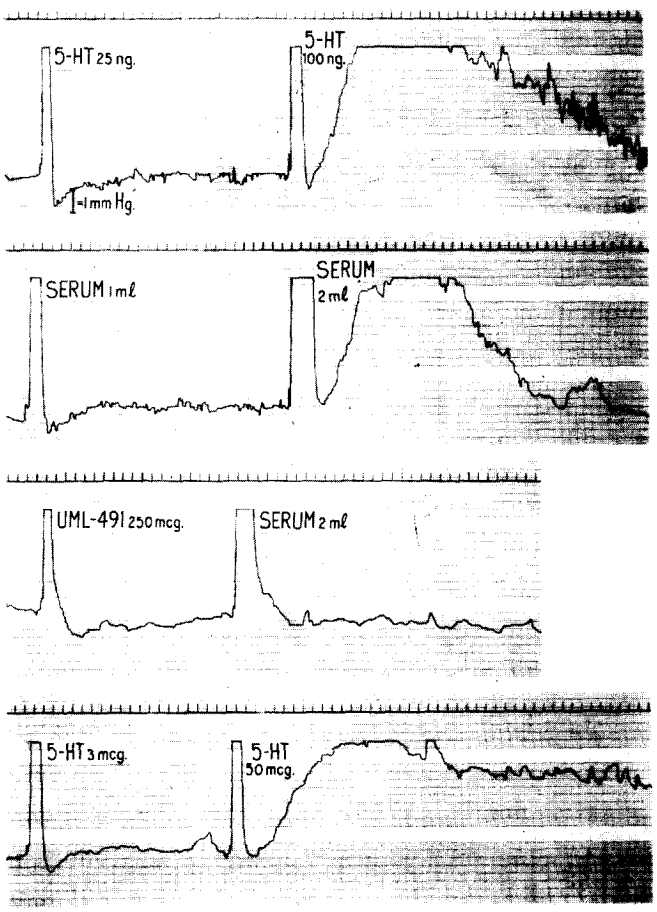

Fig. 4. 2 ml of human serum provoke the same venoconstriction as 100 ng of 5-HT. UML-491 inhibit the serum effect.

Plasma poor in platelets is not able to cause spasm of the vein. Post-ischemic plasma constricts the vein; venoconstriction from post-ischemic plasma is completely inhibited by antiserotonin drugs.

4) In four of the nine patients treated with phenelzine (75 mg per day for 10 days) and in six of the eleven patients treated by oral pargyline (50 mg per day for 1 week), an increase (from 5 to 30 fold) of sensitivity to 5-HT was noticed. In the same patients hypersensitivity to noradrenaline and adrenaline appeared (Fig. 5).

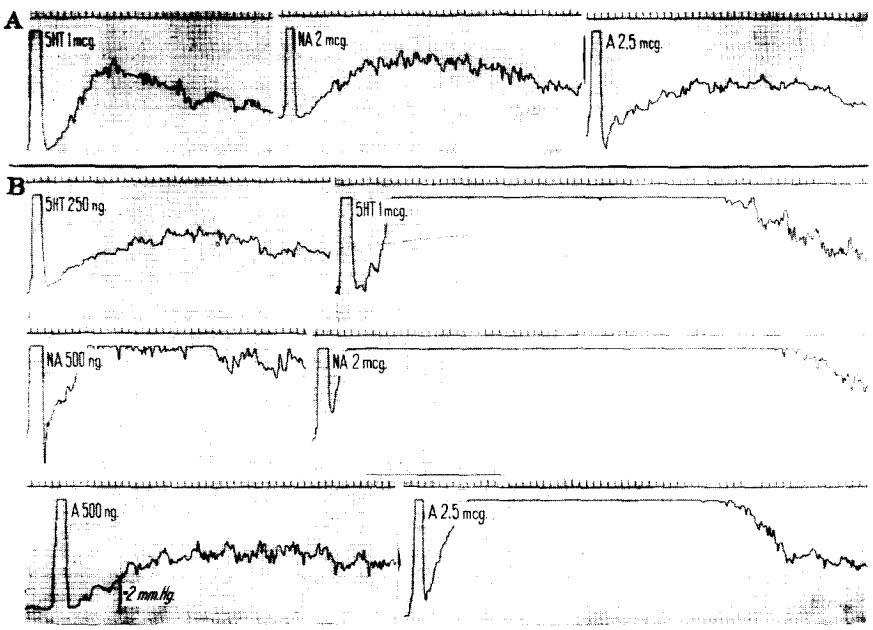

Fig. 5. Potentiation of directly acting amines (5-HT and catecholamines) after oral pargyline treatment (50 mg/day for 10 days). A) The venoconstrictor threshold effect of 5-HT, NA and A before treatment. B) After treatment.

5) Lysergic derivatives such as LSD-25, BOL-148 and UML-491 antagonize the 5-HT venospasm: the inhibition is obtained either when the drug is administered before, or is mixed with 5-HT (Fig. 6). Even when the venoconstriction has started,

antiserotonin promptly resolves the venospasm. Also drugs such as nicergoline, cyproheptadine and MY-25 inhibit the 5-HT venospasm. According to our results an approximate scale of the antiserotonin activity of these drugs may be schematized as

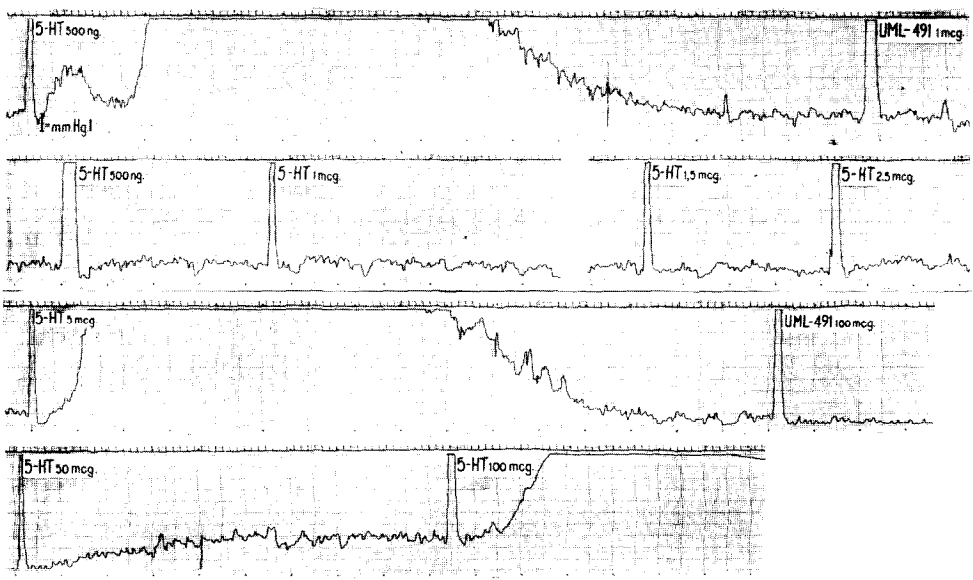

Fig. 6. Local antiserotonin activity of methysergide (UML-491). In the final test 100 mcg of UML-491 are able to inhibit an amount of 5-HT equal to 100 times the threshold dose.

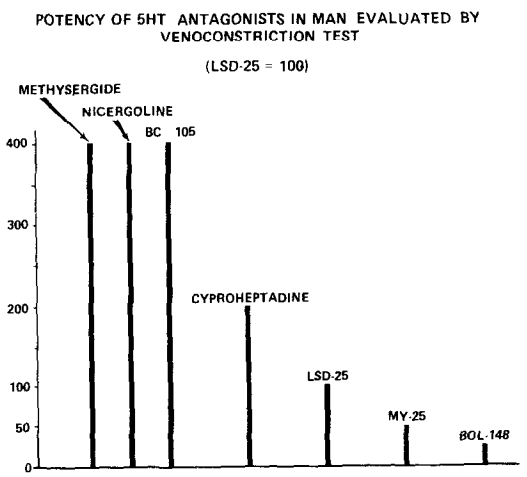

Fig. 7.

follows: UML-491 = BC-105 = nicergoline> cyproheptadine> LSD-25> MY-25> BOL-148 (Fig. 7).

6) Chronic oral administration of reserpine (0.50 mg/day for 1-2 weeks) induces in five of the seven subjects, a significant (2-3 fold) increase of the sensitivity to NA and 5-HT. In two of the seven subjects no changes were observed.

7) Oral treatment with fenclonine (0.8 g/day for 1-2 weeks) induces in four of the six patients, an increase of sensitivity to 5-HT but not to NA and A (Fig. 8). In one case the sensitivity, increased from 200 ng to 10 ng of 5-HT.

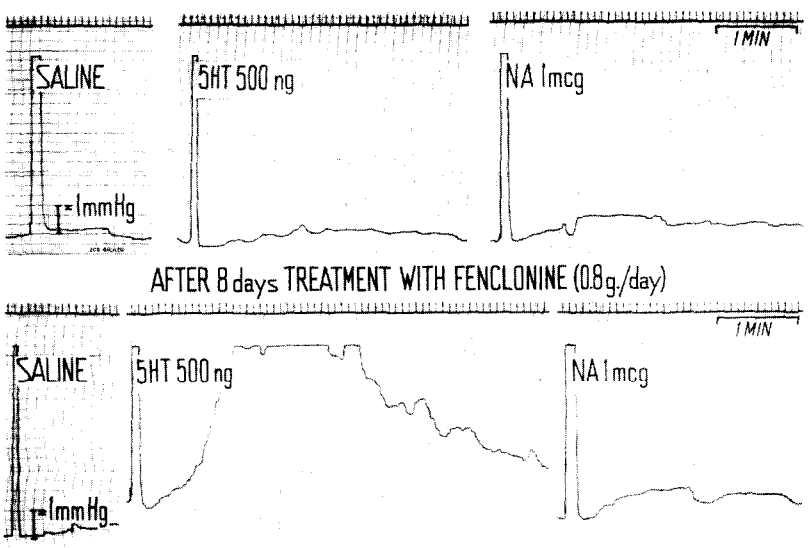

Fig. 8. 500 ng of 5-HT, are unable to constrict the vein before treatment with fenclonine (above), after the treatment the same dose is efficient (below). NA sensitivity does not appear to be increased by fenclonine.

COMMENT The most important requirement of the direct pharmacological test in man is that of the experimental economy, that is a maximum of information with a minimum of trouble to the volunteer. The VCT possesses this requirement; with little

trouble to the subject it is able to give information with satisfactory sensitivity and reproductivity. As regards 5-HT, the VCT represents the only method towards an acceptable clinical pharmacological evaluation of the specific reactivity to this monoamine in man. The sensitivity to 5-HT in normal subjects varies noticeable from one subject to the other, and this does not seem to be due, or at least only partially, to the caliber of the vein: actually it has been possible to notice hypersensitivity in small veins and vice versa.

As has been noticed, scarce variations of the sensitivity in the same subject are observable from day to day.

The human serum, according to the most recent researches, contains a vasoconstrictor substance, that is neither serotonin, catecholamines nor any other known substance (Horwitz and Mashford, 1969). Using the VCT, one may observe that 1-3 ml of human serum constrict the vein; in view of the inhibition from methysergide this effect seems correlated to 5-HT. The plasma obtained from the blood, handled carefully to avoid a release of 5-HT from the platelets, does not constrict the vein; the plasma obtained from blood sampled from the cubital vein, immediately after a few minutes of tourniquet ischemia, will exhibit a vasoconstrictor activity (Sicuteri, 1968). This venoconstrictor activity of post-ischemic plasma seems due to a release of 5-HT from platelets following circulatory arrest; in fact antiserotonin is able to antagonize the venospasm by post-ischemic plasma. By using a fluorimetric technique on dogs, an increase of 5-HT in plasma during post-ischemic hyperemia is observed (Horton, 1964). The VCT appears particularly suitable to evaluate the activity of antiserotonin drugs. The antiserotonin drugs, which exhibit a good therapeutic activity in migraine (Sicuteri, 1959; 1968), such as methysergide, BC-105, nicergoline and cyproheptadine, display a high 5-HT antagonism in animal and in man; MY-25 with poor vaso-

constrictor activity shows, in man, both a noticeable 5-HT antagonism and migraine prophylactic activity. When administered in sufficient amounts into the test vein, the antiserotonin drugs are able to inhibit doses of 5-HT 50-100 times the threshold doses.

The 5-HT potentiation from treatment with monoamine oxidase inhibitors deserves a special comment. This potentiation compared also to catecholamines varies noticeable from one subject to the other; it has been observed following local, and to a minor extent following general treatment, both with phenelzine and pargyline (Sicuteri et al. 1965).

At the time of our researches, such an important increase in sensitivity to directly acting monoamines following an MAO treatment have not been observed (Griesemer et al. 1953; Zweifach and Cascazano, 1959; Pletscher and Gey, 1961; Yeh, 1963; Elis et al. 1967; Rand and Trinker, 1968), or if so, to an uncertain or modest degree (Horwitz et al. 1960).

Surprisingly VCT indicates in some cases a striking increase of the vascular sensitivity to 5-HT and catecholamines: this contrast with previous results may be due in part to the sensitivity of the substrate. These results have been confirmed by Fishbach et al. (1966) and indirectly by Trendelenburg et al. (1970) by evaluating the influence of monoamine oxidase inhibitors on the noradrenaline uptake in perfused isolated rabbit hearts. The potentiation of the venospasm from directly acting amines stresses the possibility of a secondary supersensitivity to 5-HT, NA and A also at the level of other vascular (arterial) and non-vascular (nervous) receptors; this may contribute to the explanation of some aspects of the long and lasting hypertension from tyramine or from other indirectly acting amines, and also of psychic stimulation during pharmacological monoamine oxidase inhibition. In fact, the long lasting arterial hypertensive reaction induced by tyramine and other indirectly acting amines, may be due not only to a large release of nor-

adrenaline and adrenaline from the storage sites according to the conventional interpretation, but also to a secondary supersensitivity of vasomotor receptors to catecholamines.

The enhanced reaction to NA and 5-HT following chronic treatment with reserpine may be accepted in terms of supersensitivity similar to that of denervation: of particular significance is the increase of the 5-HT vein reactivity (NA remaining unchanged), after treatment with fenclonine (Sicuteri et al. 1973), a selective inhibitor of the serotonin synthesis (Koe and Weissman, 1966): this may be considered as a phenomenon of supersensitivity by pharmacological depletion of the agonist.

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