regular burst firing pattern induced by hexobarbital (figure 2, Ib), the synchronization level of cell discharges seems to be lower under harmaline: In the course of the harmaline action, the same unit may change its discharge pattern from irregular firing (figure 2, Ih_1) to silent periods (figure 2, Ih_2) or to a regular burst firing pattern (figure 2, Ih_3). When spikes of 2 different cells are recorded simultaneously (for example: figure 2, Ih_3), the level of the harmaline-induced synchronization seems to be different for each unit.

Autocorrelation functions of NR-unit activities obtained from periods of highly synchronized cerebellar rhythms show that harmaline does induce NR-cells to fire rhythmically (figure 2, IIH; compare also C^+ and H^+ of this figure); these discharge patterns, however, never reach that uniform regularity which can always be observed under the influence of barbiturates (compare B^{++} and H^{++} in figure 2, II).

It has been suggested[5] that the NR-rhythm induced by barbiturates, and other agents acting like barbiturates, is formed by synchronous membrane oscillations of many NR-units, which is due to their synchronized cerebellar input. Additional findings[6] have indicated that a positive correlation exists between the synchronization level of NR-cells and the amplitude of NR-rhythm. This assumption also seems to be supported by the present experiments which show low synchronization levels of cell discharges and unstable low-amplitude rhythms in the NR under the effect of harmaline.

The harmaline-induced rhythmic olivary impulses reach the spinal cord via the cerebellar cortex, fastigial and reticular nuclei[13]. Along this pathway, neurons were found to discharge in rhythmic sequences and in correlation with the tremor frequency. The cerebellar input via interposed nuclei to the NR seems to be only partly involved in these rhythmic discharges, since low synchronization levels of NR-neurons have been found to co-occur even with periods of highly regularized cerebellar rhythms. This conclusion is in accordance with that of Lamarre et al.[13] who proved that structures in the brain stem rostral to the pons are not essential for the induction and maintenance of the harmaline-induced tremor.

According to other authors[13,14], harmaline induced a synchronization of Purkinje cell activity mainly in the median area of the cerebellar vermis; the synchronization decreases rapidly with increasing distance from the midline. On the other hand, those Purkinje cells which project to the NR, via interposed nuclei, are known to be located preferentially in the intermediate zone of the vermis[15,16]. Thus, the cerebellar area projecting to the NR will be only marginally affected by the synchronizing action of harmaline. This may explain the low degree of synchrony between the electrical activities of the CB and NR under the influence of harmaline.

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Metaraminol uptake by human thrombocytes: A poor model for neuronal noradrenaline uptake

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Summary. The IC_{50} of a number of antidepressants and related drugs on the uptake of l-metaraminol and serotonin into human thrombocytes, and of noradrenaline and serotonin into rat midbrain synaptosomes were compared. In accordance with previous reports, it was found that platelets provide a good model for the study of neuronal uptake of serotonin. Platelet uptake of l-metaraminol, although correlated to some extent with noradrenaline uptake into synaptosomes, seems to be an unsatisfactory model for the neuronal uptake of the latter amine.

The uptake of serotonin (5-HT) into thrombocytes is a good model of the neuronal re-uptake of this amine[1-3]. This is especially important for the study of the inhibitory effects of antidepressants on 5-HT uptake in man, where the neuronal uptake of this transmitter cannot be estimated directly.

However, many antidepressant drugs inhibit the neuronal re-uptake of noradrenaline (NA)[4-7]. According to the catecholamine hypothesis of depression[8], it is this property of the tricyclic drugs which is crucial for their antidepressants effects[7,9].

Blood platelets take up catecholamines, especially NA, slowly and only to a limited extent[10,11], and are therefore not well suited as a model for the study of the inhibition of neuronal re-uptake of these amines[2]. It has recently been suggested that metaraminol (MR), which can be substituted for NA in the study of its uptake in sympathetic nerves[12-14], could serve a similar purpose in the platelet model[15], though very little evidence was adduced to support this theory. Since it would be a great advantage to have a model for the study of NA uptake in man, we have therefore investigated the suitability of metaraminol uptake into platelets for this purpose in more detail.

Materials and methods. C-49802-B-Ba (1-(1-methyl-amino-2-hydroxy-3-propyl)-dibenzo[b,e]bicyclo[2,2,2]octadiene-HCl) and CGP 6085 A (4-(5,6-dimethyl-2-benzofuranyl)piperidine-HCl) are experimental drugs synthesized in the laboratories of CIBA-GEIGY which selectively inhibit the uptake of NA and 5-HT respectively inhibit the uptake of NA and 5-HT respectively (manuscripts in preparation).

The uptake of ^{3}H-5-HT (12.5 Ci/mmole, Radiochemical Centre, Amersham, England) into human thrombocytes and of ^{3}H-5-HT and ^{3}H-NA (4–6 Ci/mmole, Radiochemical Centre) into rat midbrain synaptosomes was determined as described earlier[16]. The uptake of ^{3}H-MR (dl-^{3}H-MR, 6.5 Ci/mmole, New England Nuclear, Boston, Mass.) was determined by a modification of the procedure for 5-HT[16]. 0.1 ml of a 2.4×10^{-4} M solution of l-MR (Merck, Sharpe and Dohme N.V., Haarlem, Holland) containing 5 μCi/ml dl-^{3}H-MR was added to 1.1 ml platelet-rich plasma pre-incubated for 5 min with the drugs to be tested, and incubation was continued for a further 45 min. The tubes were cooled in ice, the platelets sedimented by centrifugation at $3{,}000 \times g$ for 10 min, the supernatants discarded and the pellets resuspended in 1 ml ice-cold 0.9% NaCl. The platelets were spun down again and the

supernatant discarded. The platelets were lysed by adding 1 ml H_2O and homogenizing with a glass rod on vortex mixer. Aliquots were counted together with 10 ml Instagel® (Packard Instr. Co. Inc. Downers Grove, Warrenville, Ill.). Blanks were made by incubating plasma with ^{3}H-MR at 0 °C.

IC_{50} values of antidepressant drugs with respect to MR uptake into platelets were plotted against those relating to NA uptake into rat midbrain synaptosomes. For comparison, IC_{50} values of some drugs for 5-HT uptake into platelets and synaptosomes were also plotted. Linear regressions were calculated by the least squares method.

Results and discussion. There was a highly significant positive between the inhibition of 5-HT uptake into platelets and into synaptosomes (figure 1). The correlation coefficient of 0.94 ($p < 0.001$) was similar to that reported for the correlation between 5-HT-uptake inhibition in rabbit platelets and rat brain synaptosomes (0.973)[3]. The slope of the regression line was approximately 1, which demonstrates the equivalence of the platelet and synaptosome models in evaluating the potencies of drugs affecting neuronal 5-HT uptake, as suggested by various other authors[1–3].

The time course of MR uptake was linear up to 60 min. In subsequent experiments, an incubation time of 45 min was chosen. The K_m was about 5×10^{-5} M, which compares well with the figure of 2×10^{-5} M computed from the data of Ahtee and Saarnivaara[15].

The correlation between the inhibition of MR uptake into platelets and NA uptake into synaptosomes was rather poor, although significant ($r = 0.76$, $p < 0.01$; figure 2). Moreover, the slope of the correlation line was only about 0.2; this means that drugs differing considerably in their ability to inhibit neuronal NA uptake show only slight differences with respect to inhibition of MR uptake into platelets, which may even be blurred by the experimental error. This result has a bearing on biochemical studies in humans treated with uptake inhibitors, under which conditions drug effects on 5-HT uptake can be nicely measured in the plasma before, during and after treatment[17].

Even if the high concentrations of drugs – unlikely to be reached in the plasma after treatment – necessary to inhibit MR uptake into platelets could be reduced by using a lower substrate concentration in the incubation medium, as shown to be the case for 5-HT[3], it would still not alter the fact that drugs varying widely in their ability to inhibit NA uptake show only slight differences in their effects on MR uptake into platelets.

In view of this lack of discriminating power, the measurement of drug-induced inhibition of MR uptake into thrombocytes does not appear to be a suitable model for the study of drug-induced inhibition of neuronal NA uptake in vitro and after drug treatment.

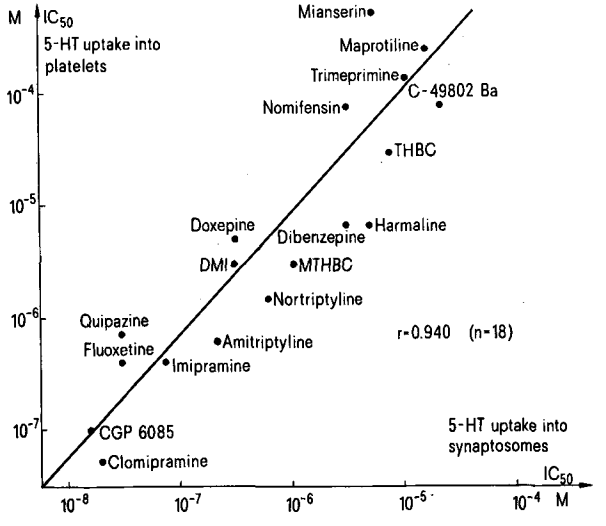

Fig. 1. Correlation between the IC_{50} of the inhibition of 5-HT uptake into human blood platelets and rat brain synaptosomes by various drugs. THBC and MTHBC are 1,2,3,4-tetrahydro-β-carboline and its 6-methoxy derivative. CGP 6085 and C-49802-Ba are experimental drugs of CIBA-GEIGY, DMI is desmethylimipramine. Linear regression was calculated by means of the least squares method.

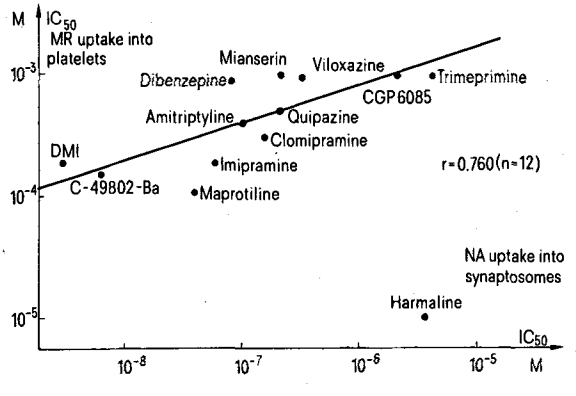

Fig. 2. Correlation between the IC_{50} of the inhibition of l-metaraminol (MR) uptake into human blood platelets and of noradrenaline (NA) uptake into rat midbrain synaptosomes by various drugs. Harmaline gave rise to a flat, atypical concentration-response curve both in platelets and in synaptosomes with respect to MR and NA uptake inhibition. The IC_{50}'s were therefore not included in the calculation of the regression line.

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