was observed between the three transport systems. The reason for this difference in action between the two agents remains unclear.

Blood ethanol concentrations ranging from 2 to 5.4 g/l are found among sober alcohol users seen in an emergency room[22], and levels as high as 7.8 g/l (about 170 mM) have been reported[23]. It has also been shown that acetaldehyde in blood ranges from 40 to 50 μM in alcoholic patients[24] and from 24 to 220 μM in the Oriental males mentioned above[21]. These observations indicate that the concentrations of acetaldehyde (50 μM) and ethanol (87 mM) used in the table are within the toxic range, but far below the lethal doses. The results presented in the table revealed that no inhibition of the 'A' system occurred at 87 mM of ethanol. This contrasts with the report by Dorio et al.[12], who described that in cultured rat liver cells the major inhibitory effects of ethanol (100 mM) exposure for 24 h were on the 'A' and 'N' systems. The discrepancy seems to be derived from the difference in methodology employed. The use of membrane vesicles has some advantages over studies at the cellular level; that is, the former provides well-defined conditions dissociated from intracellular components[13] and also makes it possible to measure the transport activity at known magnitudes and polarities of chemical or electrochemical driving forces across the membrane[13,14]. Thus, it is considered that our results are not complicated by the activities of Na^+, K^+-ATPase and protein synthesis, which are inhibited by ethanol[7,9].

Only the Li^+-dependent uptake, which was transported by the 'ASC' system, was significantly inhibited by the combination of acetaldehyde and ethanol (table). This observation is partly supported by the previous report that the plasma concentrations of alanine and serine, which are mainly transported by the 'ASC' system, are decreased by an ethanol diet in rats[25]. Thus, it is considered that acetaldehyde in combination with ethanol may add to the effects of ethanol. The selective inhibition of the 'ASC' system would appear to suggest a higher sensitivity of the transport protein and its surrounding membrane lipids to acetaldehyde-and-ethanol exposure. We speculate that the amino acid imbalance induced by the inhibition of a particular transport system may play a role in the pathogenesis of FAS in association with other factors such as zinc deficiency, the inhibition of protein synthesis and altered hormonal and neurotransmitter balance[3-5].

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Quantitative differences in the pharmacological effects of (+)- and (−)-cathinone

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Summary. The optically pure isomers of cathinone were prepared by separating synthetic cathinone racemate and used to study central and peripheral effects of these indirect sympathomimetics in rats and guinea pigs. The (−)-isomer was significantly more potent than the (+)-isomer in stimulating locomotor activity whereas no difference was observed with respect to their cardiac effects. In analogy to observations with (+)- and (−)-amphetamine such variable isomer discrimination may be due to different stereoselectivities of amine uptake mechanisms in the target tissues.

Key words. (+)-Cathinone; (−)-cathinone; cardiac stimulation; locomotor activity.

Khat leaves *(Catha edulis)* are widely used as a stimulant in East Africa and the Arab Peninsula. Most of their pharmacological effects are explained by their content of (−)-cathinone ((S)-(−)-α-aminopropiophenone), an indirect sympathomimetic with amphetamine-like centrally stimulating properties[1,2]. The actions of cathinone have been studied primarily by applying the (−)-isomer or the racemic drug. (+)-Cathinone ((R)-(+)-α-aminopropiophenone), does not occur in the plant but is obtained during chemical synthesis of the compound. It has been found to be less potent than (−)-cathinone as a stimulant of locomotor activity in mice[3,4]. However, a quantitative comparison of the central and peripheral effects of the enantiomers is lacking.

Therefore, we decided to study the potencies of synthetic (+)- and (−)-cathinone in two model systems. The locomotor activity

of rats after i.p. injection was measured to assess centrally stimulating effects. The positive inotropic and chronotropic effects on isolated guinea pig auricles were measured to study indirect sympathomimetic activity in a peripheral tissue.

Materials and methods. (±)-cathinone was synthetized as described by Schorno and Steinegger[5] and Schorno[6]. The optical isomers were separated according to Schorno[6] and Takamatsu[7]. Locomotor activity was studied in Swiss albino rats (SIV-Z strain, mostly female) weighing 219 ± 3.5 g. Following i.p. application of the drug, individual animals were placed in a circular running cage where the locomotor activity could be monitored automatically by an optical system. The cage was designed for CNS-drug testing and was made available to us by the Wander drug Company in Bern. It consisted of two concentric plastic/metal cylinders with six small openings aligned to allow the passage of light (fig. 1). Three pairs of light sources (shining dim red light) and corresponding sensors were arranged in equal distances around the cage. A signal was generated and counted electronically any time the animal crossed one of the light beams. Two such cages were combined, isolated from each other, within a sound-and-lightproof wooden case such that the treated animal and the control could be studied simultaneously. The observation period was terminated after 160 or 180 min when drug-induced hypermotility had largely subsided. To avoid racemization in solution we dissolved the optical isomers of cathinone immediately prior to their application. The injected volume was kept constant at 0.5 ml. Controls received the same volume of saline.

Inotropic and chronotropic responses to (+)-and (−)-cathinone were measured in left and right guinea-pig atria, respectively. The atria were prepared and mounted essentially as described in Kuschinsky et al.[8]. Briefly, the animals were killed by a blow on the head, the hearts rapidly removed and transferred to ice-cold Tyrode solution (composition (mM): 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 11.9 $NaHCO_3$, 0.47 $NaHPO_4$, 11 glucose). Right and left atria were removed, mounted in plexiglas holders between two silver electrode wires and suspended in two separate water-jacketed organ baths. The vessels contained 50 ml of Tyrode's solution, continuously gassed with 95% O_2 and 5% CO_2 and kept at 37 °C. The left atrium was stimulated electrically with 1.5 ms pulses at a rate of 2 Hz using twice threshold voltage. The right atrium was left to beat spontaneously. The muscles were connected to a force-displacement transducer (Grass FT 03) such that isometric contractions could be measured on a Hellige HE 119 direct recorder. The resting tension was set to 0.5–0.75 g. All muscles were equilibrated in the bath for 45 min and then exposed to 10^{-5}M noradrenaline (NA) for 15 min to fill the NA stores in the sympathetic nerve endings of the tissue. Finally the atria were washed 3 times for 5 min in 50 ml Tyrode's solution. Following this pretreatment, cumulative concentration-response (C-R) curves for (+) and (−) -cathinone were established on the same preparation. Each successive C-R curve was preceded by a NA-loading period of 15 min. Control experiments showed that under these conditions individual indirect sympathomimetics did not change their K_A values during 3–4 repetitions of the C-R curve. An additional group of experiments was done on atria from reserpinized guinea pigs (5 mg/kg serpasil® i.p., 24 h prior to the experiment). Only a single C-R curve, not preceded by an NA loading period, was run on each reserpinized atrium.

C-R curves from isolated atria were analyzed with a nonlinear least squares fitting procedure (program MODFIT, published in McIntosh and McIntosh[9]) to obtain mean values for K_A, the drug concentration which caused a half maximal effect, and Emax, the maximal effect at infinite drug concentration. For the bell-shaped dose-response curves of the in vivo experiments the significance of differences between the effects of the two isomers at a given dose and time were compared using Student's t-test. p-values < 0.05 were considered significant.

Results. 1) Effects of cathinone enantiomers on locomotor activity. Figure 2 compares the dose-response curves for the effects of (+)- and (−) -cathinone and of (+)-amphetamine on locomotor activity. All three curves were obtained under identical conditions and were measured 50 min after i.p. application of the drugs. All curves are bell-shaped. Peak effects were reached with 3 mg/kg amphetamine, 6 mg/kg (−)-cathinone. The absolute values of the peak effects were not significantly different. At the level of 1 and 3 mg/kg, locomotor activity with (+)-amphetamine was significantly higher, at 6 mg/kg significantly lower than with (−)-cathinone. At 3 and 6 mg/kg the activity with (−)-cathinone was significantly higher than with (+)-cathinone. The decrease in locomotor activity at higher drug concentrations was accompanied by symptoms of catalepsia and an increase in stereotypic low amplitude movements (chewing, gnawing, licking). It could be antagonized with the antidopaminergic drug haloperidol. This type of behavior was not quantitatively assessed in the present study. Similar bell-shaped dose-response curves have been observed for (±)- and (−)-cathinone in mice[10,11]. The maximally effective dose was 15–18 mg/kg, 2–3-fold higher than for (−)-cathinone in the present study on rats. Such dose-response curves are typical for drugs of the amphetamine type[12] which have both central NA- and dopamine-releasing actions. A (−)-cathinone-induced enhanced release of

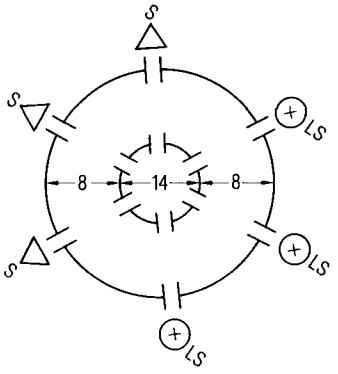

Figure 1. Cross-section of running cage used to study locomotor activity. LS = light source, S = sensor, dimensions in cm. Further explanation see text.

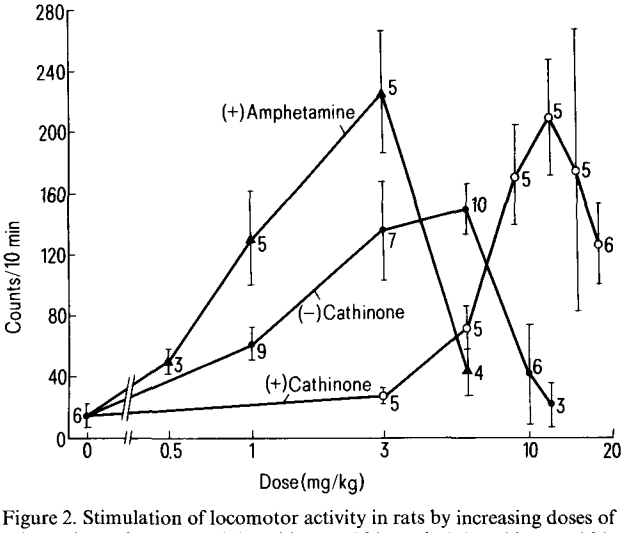

Figure 2. Stimulation of locomotor activity in rats by increasing doses of (+)-amphetamine (▲), (−)-cathinone (●) and (+)-cathinone (○). Counts (i.e. light beam interruptions) between the 45th and the 55th min after i.p. application are plotted versus dose. Error bars give (±) SEM. The number of rats tested with each dose is indicated at the individual data points.

radioactivity from rat nucleus accumbens and rabbit nucleus caudatus preparations prelabeled with ^{3}H-dopamine has been reported by Kalix[13].

Figure 3 compares the mean time courses of locomotor activity following a single i.p. application of maximally effective concentrations of the three drugs. The rate of onset of the stimulatory effect was similar and reached a peak value within about 50 min. The effect decayed somewhat more rapidly after (+)-cathinone than after either (−)-cathinone or (+)-amphetamine. In our experiments, both onset and decay of drug-induced motor activity were faster than those observed in rats by Kalix[14], probably because the time course of overall motoric stimulation is dose-dependent; in our hands, relatively high doses such as those used by Kalix (e.g. 10 mg/ kg (−)-cathinone hydrochloride) while rapidly inducing stereotyped behavior, initially had little effect on large amplitude motor activity, but caused a delayed increase in locomotion after 60–120 min.

2) Cardiac effects of cathinone enantiomers. In a second series of experiments we have compared the indirect sympathomimetic activity of the two isomers of cathinone in isolated atria from guinea pig hearts. (−)-Cathinone has been shown earlier to release radioactivity from rabbit atria prelabelled with ^{3}H-NA (16). Normalized C-R curves for the positive inotropic and chronotropic effects in NA-loaded atria are shown in figure 4. Surprisingly, the two optical isomers had almost identical K_A values: Contractile force and spontaneous beating rate were half-maximally stimulated by about 7×10^{-7} and 3.2×10^{-6} M (−)- or (+)-cathinone. Maximal contractile force reached 0.23 ± 0.02 and 0.21 ± 0.01 mN/mg wet weight in the presence of (−)-and (+)-cathinone compared to 0.09 ± 0.01 mN/mg in controls. The spontaneous frequency of right atria (123 ± 6 beats/min) was maximally enhanced to 194 ± 3 and 197 ± 4 beats/min by (−)- and (+)-cathinone, respectively. In two parallel experiments maximally effective concentrations of the standard indirect sympathomimetic drug tyramine (2×10^{-5} M) increased the concentration force from 0.09 to 0.35 mN/mg WW and the frequency from 126 to 216 beats/min.

As expected for indirect sympathomimetics, cocaine caused a parallel shift of the C-R curves to the right. Again, the shift was similar in magnitude for the two isomers of cathinone. Cocaine (6×10^{-6} M) increased the mean K_A value for the inotropic and chronotropic effects of (+)- and (−)-cathinone from 7×10^{-7} M

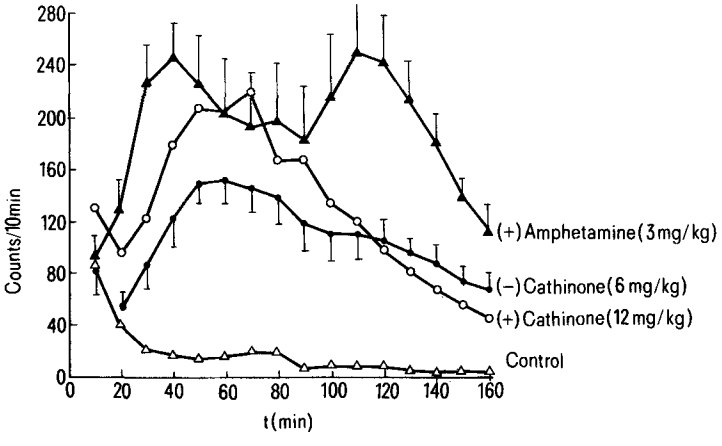

Figure 3. Time course of locomotor activity after stimulation with the maximally effective concentrations of (+)-amphetamine (▲), (−)-cathinone (●) and (+)-cathinone (○). The data points give the mean activity for consecutive 10-min periods starting 10 min after i.p. application of the drugs.

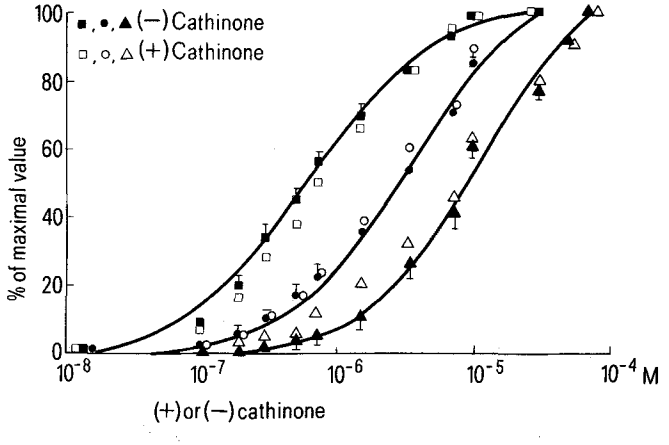

Figure 4. Concentration-response (C-R) curves for the positive chronotropic and inotropic actions of (+)- and (−)-cathinone (Cath.). The data were normalized with respect to maximal drug-induced stimulation. The curves are calculated by means of a nonlinear least square fitting procedure assuming a Hill coefficient of 1. For the sake of clarity only computer fits for the data with (−)-Cath. are shown. Error bars (SEM) are given only for the (−)-Cath. data, but were similar for (+)-Cath. Closed symbols: (−)-Cath., open symbols: (+)-Cath. ■, □ C-R curve for the positive inotropic effect, mean K_A values for (−) and (+)-Cath.: 0.64 and 0.78 μM (n = 19). ●, ○ CR curve for the positive chronotropic effect K_A values: 3.6 and 2.8 μM (n = 15). ▲, △ C-R curve for the chronotropic effect in the presence of 6×10^{-6} M cocaine, K_A values: 12.9 and 13.3 μM (N = 10).

to 3.4×10^{-6} M and from 3.2 to 13.1×10^{-6} M, respectively (fig. 4). Moreover, reserpinization of the guinea pig (5 mg/kg reserpine 24 h prior to the experiment) blocked completely any inotropic or chronotropic response to (+)- and (−)-cathinone.

Discussion. Taken together our results confirm the view that (−)-cathinone as a central stimulant is only slightly less potent than (+)-amphetamine (compare Kalix[1] and Kohli and Goldberg[15]). The (−)-isomer of cathinone was significantly more potent than the (+)-isomer. Half maximally stimulating doses of (+)-cathinone differed from the corresponding values for (−)-cathinone or (+)-amphetamine by factors of 5 and 8, respectively. On the other hand the two isomers have equivalent potencies in releasing NA from guinea pig cardiac tissue.

The reasons for the apparent difference in the stereospecificity of central and peripheral effects of cathinone, both involving catecholamine release, are not entirely clear. The most obvious possibility, rapid racemization of the isomers in the organ bath, was excluded by measuring circular dichroism to monitor the optical activity of enantiomer solutions as a function of time[6,17]. The rate of spontaneous racemization in Tyrode's solution was less than 8% within the 4-h period between the preparation of the experimental solution and the end of a single experiment. Differential stereoselectivity for various effects of (+)- and (−)-amphetamine have been attributed to differences in the stereoselectivity of neuronal amine uptake mechanisms in different parts of the nervous system. Taylor and Snyder[12] observed a 10-fold higher potency of (+)-over (−)-amphetamine in stimulating rat locomotor activity, but only a 2-fold difference in eliciting compulsive gnawing behavior. In keeping with these differential stereoselectivities of the motoric effects, (+)-amphetamine was much more potent than the (−)-isomer in inhibiting in vivo NA uptake into various brain regions, except for the striatum where the amine uptake mechanism showed little stereoselectivity[12].

It has also been reported that the preference for (+)-amphetamine in CNS excitatory effects is not seen in cardiovascular actions where (−)-amphetamine seemed to be somewhat more potent than its (+)-isomer[18]. The stereoselectivity of central and peripheral effects of cathinone has not been compared previously. However, it is known that peripheral NA storage sites are more sensitive than central dopamine sites to the releasing action of (−)-cathinone[16]. Moreover, interspecies variations of neuronal membrane transport[19] or of substrate stereoselectivity in metabolic reactions[20] are also known for (+)- and (−)-amphetamine and could provide alternative explanations for the differential effects of cathinone isomers in heart and brain.

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A possible metabolic role for *o*-diphenoloxidase in *Mycobacterium leprae*

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Summary. Among mycobacteria, *Mycobacterium leprae* is unique in its ability to oxidize a variety of diphenols to quinones in vitro. What physiologic role *o*-diphenoloxidase has in the organism remained unknown. Reducing substrates like NADPH, NADH and ascorbic acid reacted with the quinone formed from dopa (3,4-dihydroxyphenylalanine); the substrates were oxidized and the quinone was reduced back to diphenol in the process. Since the quinone undergoes reversible oxidation-reduction, diphenoloxidase might serve as an alternative respiratory mechanism in *M. leprae* for the utilization of other substrates, as has been reported in plants.
Key words. Mycobacterium leprae; o-quinone; reversible oxidation-reduction.

Mycobacterium leprae is an obligate intracellular parasite; all attempts for over a century to culture the organism in vitro have failed so far. The only specific metabolic activity detected in *M. leprae* is diphenoloxidase (EC 1.10.3.1), which converts 3,4-dihydroxyphenylalanine (dopa) and a variety of other diphenols to quinones. This activity has not been found in any other mycobacteria[1,2]. In substrate-specificity and in the effect of inhibitors, diphenoloxidase of the organisms was distinct from that in vertebrate melanocytes. It is known that in the human host *M. leprae* multiplies at sites such as the skin, peripheral nerves, adrenal medulla and the eyes where dopa or its derivatives occur.

As yet, there has been no direct experimental evidence to attribute a physiologic role for diphenoloxidase in *M. leprae*. The enzyme has been reported to function as an alternative respiratory mechanism in plants[3,4]. The quinones formed in the reaction undergo reversible oxidation-reduction and function as electron carriers; other sustrates are oxidized in the process and the quinone gets reduced back to diphenol.

$$\text{Diphenol} \xrightarrow{\text{enzymatic}} o\text{-quinone} \xrightarrow[\text{or nonenzymatic}]{\text{enzymatic}} \text{melanin}$$

o-Quinone + reducing substrates
→oxidized substrates + diphenol