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The effect of phenylpentenyl-khatamines on the release of radioactivity from rat striatal tissue prelabelled with [^{3}H]dopamine

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The CNS-stimulating properties of leaves of the khat shrub (*Catha edulis*, Celastraceae) are presumed to be due mainly to (−)-cathinone, a phenylpropylamine alkaloid that has been shown to have an amphetamine-like releasing effect at physiological catecholamine storage sites. Recently, several phenylpentenylamine alkaloids have been identified in khat leaves, and these have been evaluated, in-vitro, in the present study for their ability to induce release of radioactivity from [^{3}H]dopamine-prelabelled rat striatal tissue. It was found that the phenylpentenylamines have a weak releasing effect, and are therefore considered unlikely to play an important role in the stimulating properties of khat leaves.

The chewing of leaves of the khat shrub (*Catha edulis*, Celastraceae), a common practice in several countries of East Africa and the Arab peninsula, produces a degree of CNS stimulation characterized by moderate excitation and euphoria as well as sympathomimetic effects reminiscent of those induced by amphetamine (Eddy et al 1965; Halbach 1972).

The effects of khat are considered to be due to its content of the alkaloids (−)-cathinone (II), (+)-norpseudoephedrine (IV), and (−)-norephedrine (VI), with (−)-cathinone mainly responsible for the CNS stimulation. This alkaloid has been shown to have pharmacological properties closely resembling those of (+)-amphetamine and to be almost as potent. Furthermore, it has been possible to reproduce most of the effects of khat consumption in man by administering (−)-cathinone to animals (Advisory Group 1980). It has also been demonstrated that (−)-cathinone has an amphetamine-like releasing effect at physiological catecholamine storage sites, a finding that led to the assumption that cathinone and amphetamine have the same mechanism of action (Kalix 1984).

A few years ago, it was postulated that khat leaves contained an analogue of cathinone in which the ethylamine fragment was attached to a cinnamoyl, rather than to a benzoyl, group (see I vs II (Szendrei 1980)). It has recently been confirmed that this cinnamoyl compound is present in khat grown in the Meru district of Kenya (Brenneisen & Geisshüsler 1985). Furthermore, it was found that khat of Kenyan origin also contains the cinnamoyl analogues of (+)-norpseudoephedrine (III vs IV) and (−)-norephedrine (V vs VI) (Brenneisen et al 1984; Brenneisen & Geisshüsler 1985). In view of the possibility that these phenylpentenyl-khatamines might also be pharmacologically similar to the phenylpropylamines present in khat and thus add to its effect, the newly identified alkaloids were evaluated in-vitro for their ability to induce release from CNS dopamine terminals compared with that of the corresponding phenylpropylamines.

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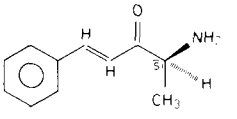
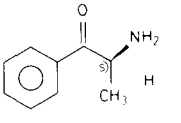
Materials and methods

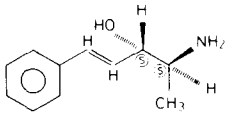
Samples of rat striatal tissue, excised from the head of the caudate nucleus immediately after killing the animals, were cut with a scalpel into cubes measuring less than 1 mm in each dimension. These were then incubated for 20 min at 37 °C in 1 mL of a solution containing (mM) NaCl 136, KCl 5·6, $NaHCO_3$ 20·0, NaH_2PO_4 1·2, $CaCl_2$ 2·2, $MgCl_2$ 1·2 and glucose 5·5, and to which 12 μCi [^{3}H]dopamine (0·8 nmol dopamine) had been added. During the incubation (as well as during the subsequent superfusion) the medium was continuously oxygenated with a mixture of 95% O_2 and 5% CO_2; its pH was 7·2. At the end of the labelling period, two of the tissue cubes were placed into each of three parallel flow cells (approx. 0·3 mL volume each) and superfused at 37 °C with the dopamine-free medium at a rate of 0·5 mL min^{-1}. During an initial washout period of 1 h, the spontaneous efflux of radioactivity from the preparations stabilized to a steady slow decrease; during the subsequent experimental period, the superfusate was collected in successive 3 min fractions. The test substances were dissolved in physio-



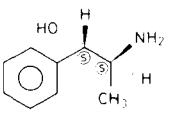
(+)-Merucathinone I



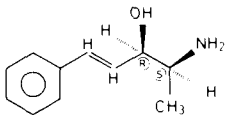
(−)-Cathinone II



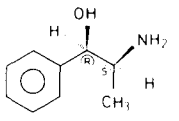
(−)-Pseudomerucathine III



(+)-Norpseudoephedrine IV



(+)-Merucathine V



(−)-Norephedrine VI

logical NaCl solution shortly before their addition to the superfusion medium, and they were added as at least 100-fold concentrates. At the end of the experiment, the tritium content of the fractions was determined by scintillation counting.

(−)-Cathinone HCl was a gift from Dr O. Braenden, United Nations Narcotics Laboratory; (+)-norpseudoephedrine HCl and (−)-norephedrine HCL were obtained from commercial sources. The phenylpentenyl-khatamines were synthesized at the Department of Organic Chemistry, University of Berne (Wolf & Pfander 1986a, b), and their identity with the naturally occurring compounds was ascertained by chromatographic and spectroscopic methods (Brenneisen & Geishüssler 1985).

Results

When rat striatal tissue that had been prelabelled with [^{3}H]dopamine was superfused with a solution containing 2 μM (−)-cathinone, a rapid and reversible increase of the release of radioactivity was observed (Fig. 1 panel A, right). A similar effect was obtained when the superfusion medium contained (+)-merucathinone. A considerably higher concentration of this alkaloid was needed, however, to produce an effect of similar amplitude. In the experiment shown in panel A (left) of Fig. 1, the effect of 2, 4 and 8 μM (−)-cathinone was found to be more pronounced than that of concentrations of (+)-merucathinone that were about 6½ times higher. In contrast, the phenylpentenylamines, (−)-pseudomerucathine and (+)-merucathine, had a releasing effect of about the same potency as the corresponding phenylpropylamines (+)-norpseudoephedrine and (−)-norephedrine (Fig. 1, panels B and C).

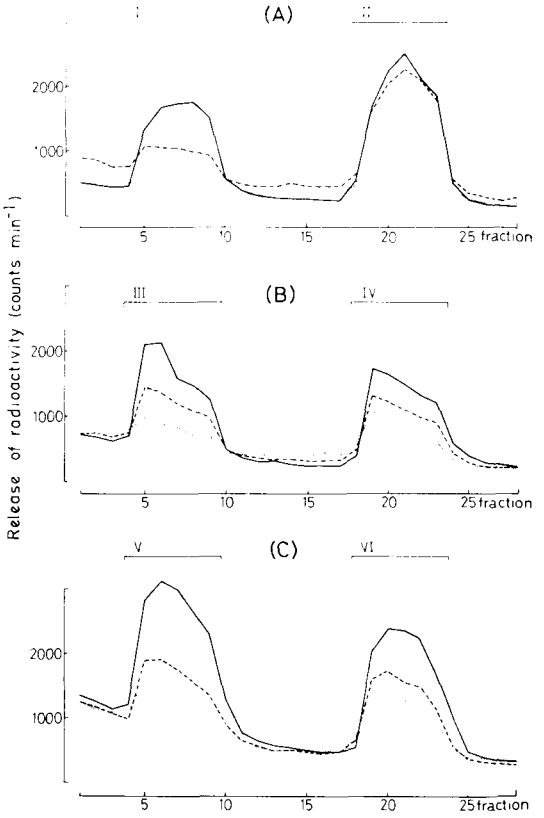


FIG. 1. The effect of various khatamines on the release of radioactivity from rat caudate nucleus tissue prelabelled with [^{3}H]dopamine. For each experiment, two tissue samples were superfused for a 15 min period with one of the phenylpentenylamines and, after an interval of 27 min, for a 15 min period with the corresponding phenylpropylamine; each fraction represents 3 min of efflux. Panel A: (+)-merucathinone (I, 13·3/26·7/53·3 μM) compared with (−)-cathinone (II, 2/4/8 μM); panel B: (−)-pseudomerucathine (III, 18/36/72 μM) compared with (+)-norpseudoephedrine (IV, 18/36/72 μM); panel C: (+)-merucathine (V, 18/36/72 μM) compared with (−)-norephedrine (VI, 18/36/72 μM). The continuous line shows the highest concentration tested and the dotted line the lowest concentration tested. The three experiments shown in each panel were run in parallel; the panels show typical sets of experiments representative of several analogous observations.

Discussion

It is well established that the CNS effects of amphetamine are primarily due to release of catecholamines from presynaptic storage sites (Groves & Rebec 1976), and it has been suggested that the stimulant effect of the khat alkaloid (−)-cathinone is also mediated by this mechanism (Kalix 1984). The possibility that the recently identified phenylpentenyl-khatamines might also be capable of inducing release of radioactivity from dopamine-prelabelled CNS tissue was investigated in-vitro using caudate nucleus of the rat, since this tissue has a high density of dopaminergic terminals and is known to play a role in psychostimulant effects. The drug-induced release of neurotransmitter from rat striatum is well documented and, in the case of dopamine-prelabelled tissue, the identity of the released material has been ascertained (Liang & Rutledge 1982).

(+)-Merucathinone was found to be of low potency in inducing release as compared with (−)-cathinone. This seems to suggest that (+)-merucathinone does not play an important role in the psychostimulant effect of khat leaves. Nevertheless, (+)-merucathinone is a molecule that is more lipophilic than (−)-cathinone, and thus could more easily penetrate the CNS. It would be of interest, therefore, to compare the kinetics and metabolism of these two khat alkaloids.

(−)-Pseudomerucathine and (+)-merucathine were found to be approximately equipotent to the corresponding phenylpropylamines which have a rather weak releasing effect. It is therefore unlikely that these two phenylpentenylamines play a major role in the stimulating effect of khat leaves. It was somewhat surprising to

find that in spite of the longer side chain, the phenylpentenylamines retained a capacity to induce release. Indeed, most compounds that act as indirect sympathomimetics have a two-carbon distance between the aromatic ring and the nitrogen. There is evidence, however, that even some aliphatic amines are indirect sympathomimetics of considerable potency (Palm & Holtz 1965).

It can be assumed that the khatamine-enhanced efflux of radioactivity is of intraneuronal origin since isoprenaline, under the conditions of the experiments described here and at concentrations up to 20 μM, had no effect on the release of radioactivity (Kalix 1986). Furthermore, (−)-cathinone and (+)-norpseudoephedrine have been found to be very effective releasing agents in rabbit heart tissue (Kalix 1983), even though in rabbit heart the extraneuronal catecholamine uptake system is poorly developed (Graefe et al 1978). On the other hand, it might be argued that the increase of release observed in the present experiments was due to inhibition of reuptake rather than to increased efflux from presynaptic storage sites. This is not very likely, however, since under the conditions of the present experiments the prototype reuptake inhibitor cocaine produces, at a concentration of 5 μM, only a very weak increase in the release of radioactivity (Kalix 1986).

Although in-vivo experiments are necessary before any definitive statement can be made, it does not seem likely that the phenylpentenylamines contribute substantially to the psychostimulant effect of khat leaves, not only because of their relatively low potency in inducing release of radioactivity from catecholamine sites, but also because their concentration in khat leaves is lower than that of the corresponding phenylpropylamines. It is quite possible, that the phenylpentenylamines have pharmacological actions other than CNS stimulation, for example effects on the cardiovascular system. (−)-Cathinone and (+)-norpseudoephedrine have been shown to be not only much more potent in releasing ^3H from [^3H]noradrenaline-prelabelled heart tissue than from [^3H]dopamine-prelabelled CNS tissue, but also to be equipotent in heart tissue (Kalix 1983). It would be of interest, therefore, to investigate the possible effect of the phenylpentenyl-khatamines on the release of radioactivity from [^3H]noradrenaline-prelabelled peripheral sympathetic nerve endings.

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