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EFFECT OF THE ALKALOID (-)CATHINONE ON THE RELEASE OF RADIOACTIVITY FROM RABBIT ATRIA PRELABELLED WITH ^{3}H-NOREPINEPHRINE

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Summary

In certain countries of East Africa and the Arab Peninsula, fresh leaves of the khat shrub are used as a stimulant. The effect of the plant material can be explained by the presence of the phenylalkylamine alkaloid (-)cathinone in the leaves, since this substance has been shown to have an amphetamine-like releasing effect on CNS tissue prelabelled with ^{3}H-dopamine. Characteristically, the chewing of khat is accompanied by sympathomimetic effects, especially at the cardiovascular level. To test whether these might be due to release of neurotransmitter from adrenergic nerve endings, the effect of (-)cathinone on the efflux of radioactivity from isolated rabbit atrium tissue prelabelled with ^{3}H-norepinephrine was investigated. It was found that, at concentrations below 1 µM, (-)cathinone caused an immediate increase of efflux. The effect was dose-dependent and was potentiated by pretreatment of the rabbits with reserpine. Preincubation of the tissue with desipramine and cocaine prevented the induction of release by (-)cathinone. The results indicate that the alkaloid (-)cathinone has an amphetamine-like releasing effect on noradrenergic nerve endings and they suggest that the cardiovascular symptoms observed during khat consumption are due to release of neurotransmitter from physiological storage sites.

Among the inhabitants of East Africa and the Arab Peninsula, fresh leaves of the khat shrub (Catha edulis, Celastraceae) are widely used as a stimulant. The phenylalkylamine alkaloid (-) cathinone (α-aminopropiophenone), which has recently been isolated from the plant (1), is thought to be its active principle since this compound not only produces behavioural effects that are analogous to those of amphetamine, but is also of a similar potency (2,3,4). More important, it has recently been demonstrated that (-)cathinone, like (+)amphetamine, is capable of releasing radioactivity from CNS tissue prelabelled with ^{3}H-dopamine. This effect has been shown to occur in the caudate nucleus (5) and in the nu-

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cleus accumbens (6), two organs that are thought to mediate the central effects of amphetamine.

Characteristically, the consumption of khat is accompanied by peripheral symptoms such as transient conjunctival and facial congestion, tachycardia and hypertension, which are considered to be sympathomimetic effects of the plant material (7). Experiments were therefore performed in order to determine whether the cardiovascular manifestations observed during khat chewing might be due to a release of catecholamines from physiological storage sites, or, in other words, whether the analogy in the mechanism of action of (-)cathinone and (+)amphetamine that has been shown to exist in the central nervous system would also apply to peripheral sites.

Methods

Rabbit atrium tissue was obtained from hearts excised immediately after killing of the animals. The atria were dissected in Krebs-Ringer bicarbonate buffer and samples of approximately 0.5 mg wet weight (blotted) were removed with a scalpel.

These were then incubated for 15 min in a solution containing (mmol/liter) NaCl 136; KCl 5.6; $NaHCO_3$ 20.0; NaH_2PO_4 1.2; $CaCl_2$ 1.2; $MgCl_2$ 1.2; glucose 5.5; and then, for a further period of 15 min, in the above solution supplemented by (mmol/liter) pargyline 0.08, tropolone 0.10 and ascorbic acid 0.28. Subsequently the samples were labelled by incubation for 30 min in 1 ml of the latter solution to which 12 µC ^{3}H-norepinephrine (0.8 nmol norepinephrine) had been added. During the incubation as well as during the subsequent superfusion the medium was continuously gassed with a mixture of 95 % O_2 and 5 % CO_2, its pH was 7.3 and the temperature 37°C.

At the end of the labelling period the tissue samples were submerged for three consecutive periods of 3-5 min each in 30 ml norepinephrine-free incubation medium. Three samples were then transferred to a flow cell of approximately 0.3 ml volume, and three other samples were transferred to a parallel flow cell. Subsequently, the tissues were superfused at a rate of 0.5 ml/min with the supplemented incubation medium. After an initial washing period of 30 min the spontaneous efflux of radioactivity from the samples became stabilized and provided a steady background which was not altered by the flow stop of about 3 seconds that was necessary for switching to a modified superfusion medium. During the subsequent experimental period, the superfusate was collected in successive 3 min fractions and its tritium content was determined by scintillation counting.

The study consisted of 39 experiments, for which 44 rabbits of approximately 3 kg weight were sacrificed. Typical experiments with results representative of several analogous observations are illustrated in the figures.

Results

When low concentrations of (-)cathinone were added to the medium superfusing the norepinephrine-prelabelled heart tissue, an increase of the efflux of radioactivity from the preparation occurred within 1-2 min. The effect was perceptible at concentra-

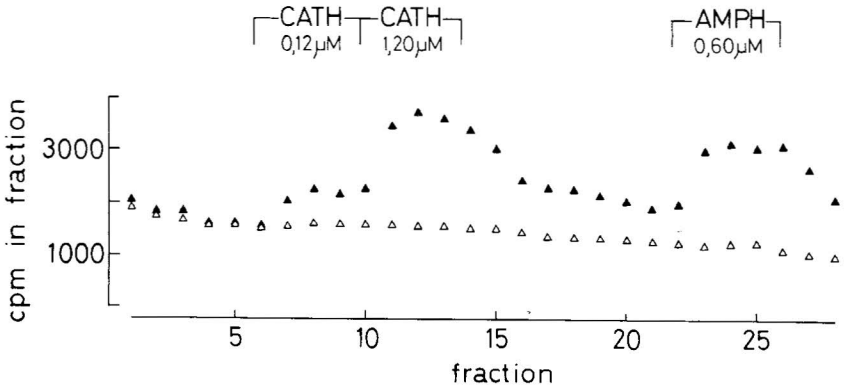

FIG. 1

Effect of (-)cathinone and of (+)amphetamine on the efflux of radioactivity from rabbit atrium tissue prelabelled with ^{3}H-norepinephrine. The efflux from a control preparation superfused simultaneously is indicated by open triangles. Each fraction corresponds to 3 min of efflux. Typical of 5 experiments.

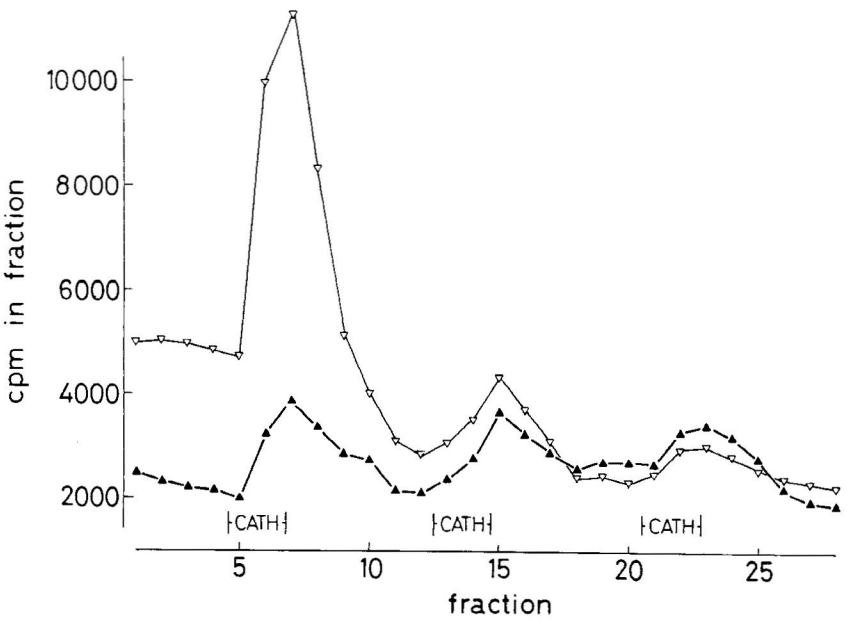

FIG. 2

Repetitive stimulation with 0.80 μM (-)cathinone of the efflux of radioactivity from rabbit atrium tissue obtained from untreated (▲) or reserpinized (▽) animals and prelabelled with ^{3}H-norepinephrine. Reserpine (Serpasil^{R}) was injected intramuscularly 20 hours before the experiment at a dose of 2 mg/kg. Typical of 4 experiments.

tions below 1 μM and was dose-dependent. Withdrawal of (-)cathinone from the medium resulted in a pronounced decrease of the efflux of radioactivity. During a subsequent test, the increase of efflux induced by (-)cathinone could be reproduced by a similar concentration of (+)amphetamine (fig. 1). The amount of radioactivity released during three repetitive exposures to a given concentration of (-)cathinone was approximately the same each time (fig. 2) and this was also the case when (+)amphetamine was used for the induction of release (not shown). However, when analogous experiments were performed with tissue obtained from rabbits pretreated with reserpine, tachyphylaxis rapidly developed. In these experiments, the effect of the first (-)cathinone dose was far greater on tissue from reserpinized animals than on tissue from untreated animals (fig. 2). Further experiments showed that it was possible to abolish the releasing effect of (-)cathinone by preperfusing the tissue samples with desipramine, an inhibitor of catecholamine uptake. Desipramine alone did not appear to modify the efflux of radioactivity from the preparation (fig. 3). Analogous results were obtained when cocaine was added to the incubation medium prior to (-)cathinone exposure. On the other hand, pre-exposure of the tissue to the potent local anaesthetic tetracaine did not affect the (-)cathinone-induced release to any noticeable extent (fig. 4). Furthermore, it was observed that in tissue from reserpinized animals as well, desipramine and cocaine abolished the releasing effect of (-)cathinone.

Discussion

The widespread use of khat leaves as a stimulant and the resulting public health problems have lead to a considerable interest in the pharmacology of the recently discovered khat alkaloid (-)cathinone (2,3,4,13,14). Since khat consumption is accompanied by certain peripheral symptoms, especially at the cardiovascular level, it seemed of interest to investigate whether these might be the consequence of an indirect sympathomimetic effect of (-)cathinone, that is whether the alkaloid is capable of inducing release of neurotransmitter from adrenergic nerve endings. The rabbit atrium was chosen for these experiments not only because of its relevance to the cardiovascular status but, more important, because the drug - induced efflux of neurotransmitter from this tissue has been well characterized (8). The rabbit heart has a further advantage in that non-neuronal effects appear to be negligible, since the extra-neuronal catecholamine uptake system of this tissue is poorly developed (9).

In the present study it was found that the khat alkaloid (-)cathinone can cause an immediate increase of the release of radioactivity from rabbit atria prelabelled with ^{3}H-norepinephrine. The substance was observed to be highly potent, since concentrations of as low as 0.12 μM induced a perceptible rise in efflux (fig. 1). By comparison, the (-)cathinone concentrations required to enhance release from dopaminergic terminals in the CNS are at least one order of magnitude higher; threshold effects have in fact been observed with 2 μM in rabbit caudate nucleus (5) and with 3 μM in rat nucleus accumbens (6). During these latter investigations on CNS tissue, it was determined that (-)cathinone is a

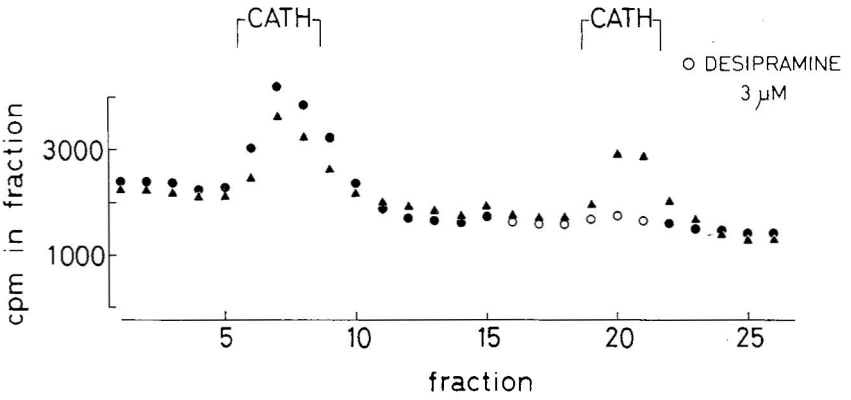

FIG. 3

Effect of desipramine on the (-)cathinone-induced release of radioactivity from rabbit atrium tissue prelabelled with ^{3}H-norepinephrine. Two preparations were perfused in parallel and stimulated twice with 1 μM (-)cathinone during a 9 min period. Before and during the second stimulation the test preparation (circles) was perfused for 18 min (open circles) with 3 μM desipramine. The efflux from the control preparation is indicated by triangles. Typical of 7 experiments.

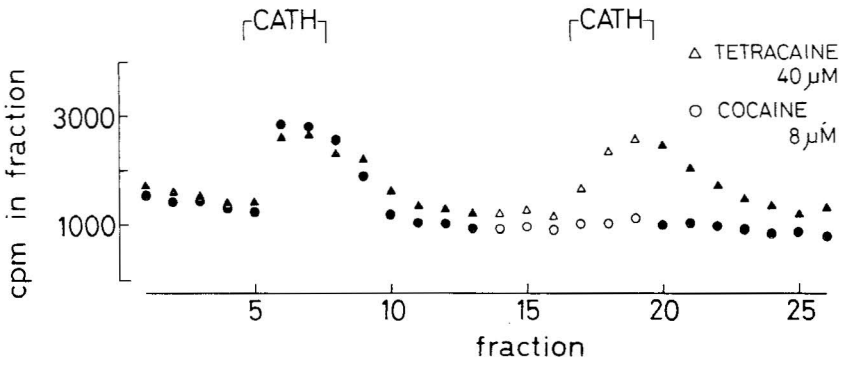

FIG. 4

Effect of cocaine and tetracaine on the (-)cathinone-induced release of radioactivity from rabbit atrium tissue prelabelled with ^{3}H-norepinephrine. Two preparations (●, ▲) were perfused in parallel and stimulated twice with 1 μM (-)cathinone during a 9 min period. Before and during the second stimulation the superfusion medium contained, for an 18 min period, either 8 μM cocaine (O) or 40 μM tetracaine (Δ). Typical of 5 experiments.

releasing agent approximately half as potent as (+)amphetamine. The experiment illustrated in fig. 1 indicates that a similar ratio of potency applies to release from peripheral noradrenergic nerve endings.

When ^{3}H-norepinephrine prelabelled atrium tissue was exposed repeatedly to the same concentration of (-)cathinone, the amounts of radioactivity egressing during (-)cathinone superfusions remained more or less constant. The non-occurrence of tachyphylaxis in these experiments is probably accounted for by the fact that the (-)cathinone concentration used was submaximal and the number of (-)cathinone applications limited. Pronounced tachyphylaxis was, however, observed when the same experiment was performed with tissue obtained from animals injected with reserpine in order to prevent the vesicular storage of norepinephrine. Furthermore, under these conditions the effect of the first (-)cathinone dose was potentiated, an observation that is agreement with that reported by Enna and Shore (10) regarding the effect of (+)amphetamine in heart tissue obtained from untreated rats or reserpinized rats and subsequently incubated in the presence of a MAO inhibitor. Although the superfusion medium used in the present experiments was supplemented with pargyline and tropolone in order to prevent the enzymatic degradation of the exogenous norepinephrine, it cannot be excluded that the label released during (-)cathinone stimulation from untreated or reserpinized tissue might represent different compounds. Nevertheless, the characteristic modification of the (-)cathinone effect by previous reserpine treatment as shown in fig. 2 is a supportive argument for the classification of the new alkaloid as an indirect sympathomimetic (11).

Under the conditions of the present experiments, desipramine and cocaine did not cause a perceptible change in the efflux of radioactivity from rabbit atrium tissue. However, both compounds were found to abolish the efflux increase caused by addition of (-)cathinone to the medium. Furthermore it could be demonstrated that this effect was unrelated to the membrane stabilizing properties of cocaine, since the effect of (-)cathinone was unaltered in the presence of rather high concentrations of the potent local anaesthetic tetracaine. As desipramine and cocaine both inhibit neuronal catecholamine transport, it can be inferred that an intact catecholamine uptake system is a prerequisite for the releasing action of (-)cathinone. Since similar observations with regard to the effect of these compounds on amphetamine-induced release have led to analogous conclusions (12), it would appear that at the level of the neuronal membrane, (-)cathinone behaves like (+)amphetamine.

Since the present study indicates that the releasing effect of cathinone on the noradrenergic neurone is, in several respects, pharmacologically analogous to that of amphetamine, the new khat alkaloid must be considered an indirect sympathomimetic. It can be presumed, therefore, that the peripheral effects of khat chewing, such as mydriasis (7) and the cardiovascular syndrome mentioned earlier, are at least partially due to the releasing action of cathinone. Thus, it seems likely that not only the khat-induced stimulation of the central nervous system (5,6), but also the peripheral manifestations observed during khat consumption involve cathinone-induced release of catecholamines from physiological

storage sites.

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