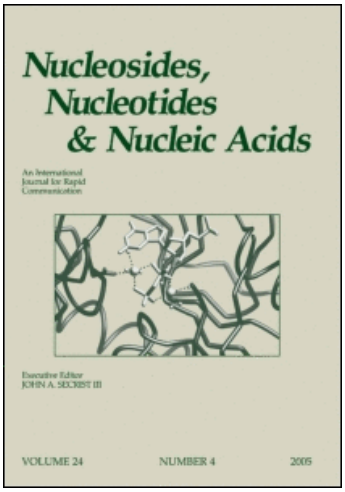

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SELECTIVE A_1-ANTAGONISTS FOR TREATMENT OF COGNITIVE DEFICITS

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Abstract. A novel compound, KFM 19, is introduced, which turned out to be a selective adenosine A_1-antagonist with good bioavailability. Its pharmacological profile suggests a high therapeutic potential for dementia and related cognitive deficits.

Introduction

Adenosine in the CNS acts as a neuromodulator exerting its effects via A_1 and A_2 receptors [1]. The majority of adenosine receptors is localized in the brain, where A_2 receptors are found in the striatum, while A_1 receptors predominate in the hippocampus and in the cortex. In general terms, A_1 receptors cause an inhibition of the release of excitatory as well as of inhibitory neurotransmitters and a postsynaptic decrease of excitability. These effects are G-protein dependent, and they are mediated by an inhibition of adenylate cyclase and of calcium influx and by an increase in potassium efflux [2]. In turn, A_1 antagonists can be expected to enhance the release of various neurotransmitters (e.g. acetylcholine) and to depolarize postsynaptic neurons. Investigations in this area so far have been hampered by the lack of availability of potent and selective A_1 antagonists which show sufficient bioavailability [3]. The compound to be presented here, KFM 19 (8-(3-oxocyclopentyl)-1.3-dipropyl-7H-purine-2.6-dione) does not

cause these problems because of its sufficiently high aqueous solubility and good bioavailability. Thus it could be thoroughly tested in vitro as well as in vivo.

Methods and Results

Adenosine receptor binding A_1 and A_2

Brain membrane homogenates were prepared from rhesus monkey cortex (A_1 receptor) or striatum (A_2 receptor) according to the methods described elsewhere [4-7]. The radioligand used was (^{3}H)-DPCPX (1.3-dipropyl-8--cyclopentyl-adenosine, NEN/Dupont, 0.5 nmol/l). For determination of non-specific binding 10^{-5}mol/l(-)-PIA ((-)-N^6-phenylisopropyl-adenosine) was used. The entire binding procedure was carried out automatically by a laboratory robot of Zymark Inc. (Idstein). K_i values were calculated on a WANG computer according to Rominger and Albert [8]. A_2 receptor binding assay was performed in the same way, except that the radioligand was ^{3}H-NECA (N- ethoxycarbonylamido - adenosine 5 nmol/l), in the presence of 30 nmol/l(-)-PIA to mask the A_1 receptor fraction of the preparation.

KFM 19 demonstrated high affinity to and selectivity for A_1 receptors from monkey cerebral cortex with K_i = 10.5 ± 2.8 nmol/l (n=6). The affinity of KFM 19 to A_2receptors in striatum homogenates was more than 100 fold lower with K_i= 1512 ± 774 nmol/l (n=4). These results, as well as those of some reference compounds, are shown in TABLE 1.

Acetylcholine determination in vivo:

The experiment was carried out in male Wistar rats (180-220g). KFM 19 (aqueous suspension, 1-30 mg/kg) or saline (controls) were administered perorally by gavage. One hour after administration, the rats were sacrificed by microwave irradiation focussed on the brain. A 7 KW microwave irradiation of 1.7 s duration caused immediate cell death without stress-induced increase of brain enzymes. Acetylcholine determination from the brain samples was carried out by a HPLC method[9].

KFM dose-dependently increased the acetylcholine content in the cerebral cortex. At a dose of 10 mg/kg, the acetylcholine levels increased to 169 ± 11% (n=20) as compared to controls. DPCPX which was tested as a reference compound at a dose of 10 mg/kg raised rat cortical

TABLE 1 Affinities to adenosine A_1 and A_2 receptors in monkey brain of KFM 19 and some reference compounds. K_i-values (nmol/l) ± S.D.

	CORTEX		STRIATUM	
	A 1		A 2	
KFM 19	10.5 ± 2.8	(n=6)	1512 ± 774	(n=4)
DPCPX	4.7 ± 2.7	(n=4)	340	(n=2)
DIPROPENYL-CPX	41.1 ± 10.4	(n=4)	370	(n=2)
CAFFEINE	48583 ± 3202	(n=4)	62282	(n=2)
THEOPHYLLINE	8982 ± 2879	(n=4)	20191	(n=2)

TABLE 2 Acetylcholine contents (% of control±S.D.) in rat cerebral cortex following p.o. administration of KFM 19.

KFM 19 (mg/kg)	1	3	10	30	control
ACH content	119±6	168±13	169±11	263±15	100±20
	n=20	n=10	n=20	n=5	n=25

acetylcholine levels to 138 ± 25% (n=5). The effects of KFM 19 at various doses are listed in TABLE 2.

Facilitation of synaptic transmission in rat hippocampal slices

Transverse hippocampal slices from adult Wistar rats were prepared according to Greene et al.[10]. The slices were placed in a recording chamber and continuously superfused by oxygenated artificial cerebrospinal fluid. Electrical stimulation of the Schaffer/commissural afferents was done via bipolar stimulation electrodes. Stimulus-evoked responses (3-5 consecutive sweeps at 100s intervals) were elicited and extracellularly recorded from CA_1 region. Their amplitudes were evaluated before and after addition of test solutions to the medium. If a single stimulus was followed by more than one population spike the individual amplitudes were added up and their sum defined as stimulus response. Statistical analysis was carried out with Student's t-test for paired observations.

Facilitation of synaptic transmission by KFM 19 was tested in hippocampal slices with a constant adenosine concentration of 3.7 X 10^{-4}M added to the superfusion medium. At this adenosine concentration, the stimulus-evoked population spike was totally suppressed in each case. KFM 19 not only fully restored synaptic transmission in a dose-dependent manner, but further increased spike amplitudes above the level of controls without adenosine pretreatment (cf. FIG.1).

Influence of adenosine agonist induced locomotor depression in mice

Male mice with a body weight of 20-30 g (strain Chbi:NMRI) were used. The adenosine agonist N-(1.2-dihydroxy-3-propyl)-adenosine (DHPA) was given to all animals in a dose of 1 mg/kg p.o., except for the control group which received saline. One hour later the drugs to be tested for adenosine antagonistic effects were given orally; control groups again received saline. Immediately after the second administration locomotor activity was recorded over a period of 60 minutes with an electronic motility measuring device (Rhema-Labortechnik, Hofheim/Ts.). KFM 19 was tested in doses of 0.3, 1 and 3 mg/kg; dipropylcyclopentylxanthine (dipropyl-CPX) and dipropenylcyclopentylxanthine (dipropenyl-CPX) were tested as references. All substances were suspended in distilled water. The results were checked for statistical significance according to Dunnett [11].

The adenosine agonist DHPA when given alone, reduced locomotor activity to approximately 10% of saline-treated controls. KFM 19 dose-dependently antagonised this effect with an ED_{50} value of 0.6 mg/kg p.o.. Three mg/kg of KFM 19 fully restored normal motility. Both reference compounds showed similar effects, however, turned out to be not as potent as KFM 19. ED_{50} values were 1.0 mg/kg for dipropyl-CPX and 2.2 mg/kg for dipropenyl-CPX.

Effects of KFM 19 on daytime locomotor activity of mice

Locomotor activity of individual female mice (20 g) of the strain Chbi:NMRI was recorded in test chambers with 5 horizontal light beams. Light beam interruptions were continuously recorded for two hours following drug, or placebo, administration. KFM 19 was given orally by gavage in 0.5% aqueous tylose solution immediately before starting the measurement. Control groups having received tylose solution were run in

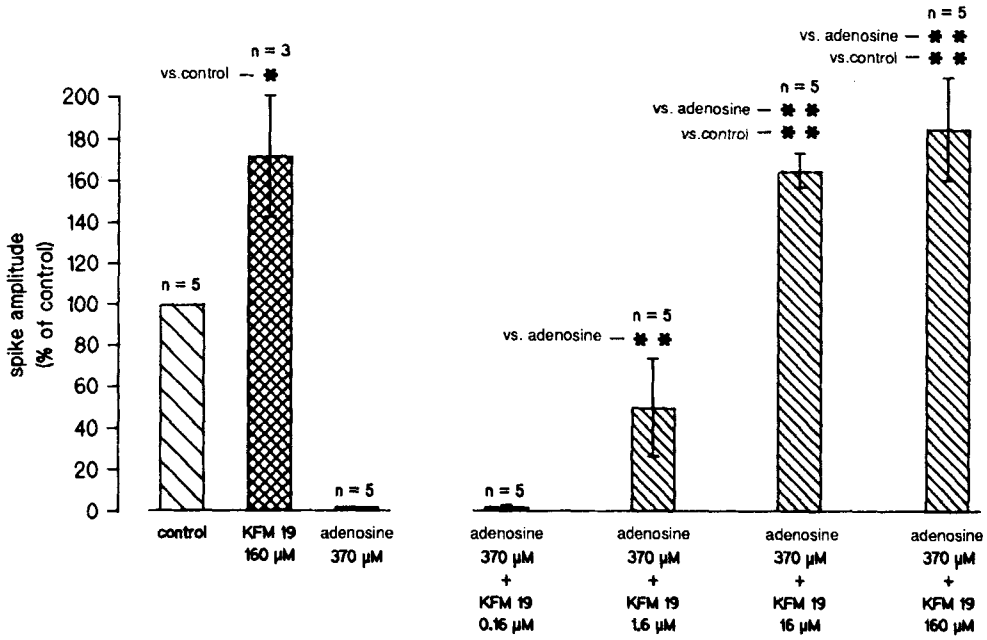

FIG.1 Effects of KFM 19 on stimulus-evoked responses in rat hippocampal slices

parallel. For calculation of statistical significance the t-test was applied.

As compared to the placebo-treated group, KFM 19-treated mice showed a slight tendency towards an increase in activity during the first hour following administration. However, this difference was statistically significant ($p<0.05$) only in the highest dose tested (40mg/kg p.o.). During the second hour after administration significant effects of KFM 19 could not be found in either dose. These results indicate a mild short-lasting stimulatory effect of KFM 19 when given in high dosages.

EEG experiments with KFM 19 in rabbits

Rabbits of either sex (body weight from 2,8-5 kg) chronically implanted with silver screws in the skull were used. Computer-aided power spectrum analysis including determination of relative power for the delta, theta, alpha and beta frequency bands was done for pre- and postadministration

periods. Test compounds were given i.v.(scopolamine 0.2 mg/kg dissolved in saline), or by gavage (KFM 19, vehicle 0.5% tylose solution). Following drug injection, the EEG was recorded for 2 hours. Statistical evaluation was done with the t-test for paired differences. Power spectra following drug injection were compared with corresponding pre - drug values.

Visual evaluation of the EEG records gave no indication of pathological patterns related to either of the KFM 19 doses. Circadian rhythmicity of vigilance remained normal. KFM 19, when given alone in doses of 1, 3 and 10 mg/kg p.o. did not significantly alter the distribution of power within the frequency bands. Thus a systematic intrinsic drug effect was not visible. Scopolamine in the dose of 0.2 mg/kg i.v. significantly increased delta-power in a typical manner, with the dominant frequency falling to values in the range of 0.3-3.5 Hz. KFM 19 pretreatment suppressed this rapid increase of delta-power and the decrease of the dominant frequency: concomitant administration of KFM 19 prevented scopolamine induced changes in rabbit EEG.

Effects on sleep-wakefulness of cats

Adult mongrel cats of either sex were used. The animals had been implanted with electrodes for EEG-, EMG- and EOG-recording. Daytime recording was performed for 6 hours following administration of either placebo or test compound. Behaviour of the animals was monitored with a video camera. The global profile including vigilance stages, active and relaxed wakefulness, slow wave and REM-sleep [12], was determined for each minute of the total recording and calculated as percent of the total recording time. Other sleep-wakefulness parameters such as sleep- and REM-sleep-latency, number, duration, and distance of REM-phases patterns were determined for each hour of the recording time. Baseline values were obtained from 5-10 sessions per individual animal following placebo treatment. KFM 19 was administered in doses of 0.1, 1 and 10 mg/kg p.o.; caffeine (30 mg/kg p.o.) served as reference.

Following the administration of KFM 19, global sleep profile showed a tendency towards a reduction of sleep (including REM sleep) periods as compared to nontreated controls. The relative proportion of REM sleep and REM sleep-latency remained constant, total sleep latency slightly increased. In the time-related profile, states of (active or relaxed) wake-

fulness predominated throughout the six hours of observation. However, these changes were moderately pronounced and significant only in the 10 mg/kg dose. In contrast, caffeine (30 mg/kg) under the same experimental conditions exerted very potent effects. Sleep and REM-sleep were totally suppressed, states of vigilance predominated throughout the observation period.

Appetitive spatial learning of rats in an automated Y maze

In the first series of experiments, learning performance of male NORWAY rats was tested in a Y maze orientating task. Groups of 3 month old animals (restricted food intake; body weight approximately 200 g) were compared with old-aged animals (24 months of age; body weight 300 g). In order to receive a food pellet the animals had to explore the three symmetric arms of the apparatus in either a clockwise or counterclockwise order and to press a lever at the end of each arm. A light was switched on when the correct arm had been entered as a secondary discriminative stimulus. Training sessions took place daily and lasted for 20 minutes. As soon as the animals showed constantly high rates of correct choices, the test was reversed so that the animals had to explore the 3 arms in the opposite way for positive reinforcement.

In a second series of experiments, male Wistar rats which weighed ca. 200 g were used in the same experimental set up. After initial training the rats were divided into three groups, two of which consequently received lesions in the nucleus basalis magnocellularis (NBM) according to standard procedures [13], whereas the third group was sham operated. Two weeks after surgery, training was continued, with the lesioned rats receiving placebo or 5 mg/kg of KFM 19, 1 hour before each session. After completion of the training experiments animals were sacrificed for histological confirmation of the lesion sites. Mean numbers of correct responses in each group were calculated and statistically analysed using a t-test.

Young rats showed a markedly better learning performance than old rats. However, the old animals pretreated with KFM 19, almost reached the response level of the young rats and showed a significantly higher rate of correct responses than the non-treated control group (cf. FIG 2a). Reversal of the spatial learning task caused a decrease in the number of correct choices in all groups. During the subsequent new

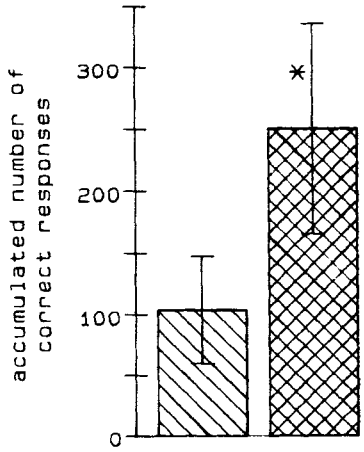

FIG.2a Differences in learning performance in old aged rats pretreated with placebo (left bar, n=5) or KFM 19 (5 mg/kg p.o., right bar, n=5). Number of correct responses was accumulated from a 2 week training period.

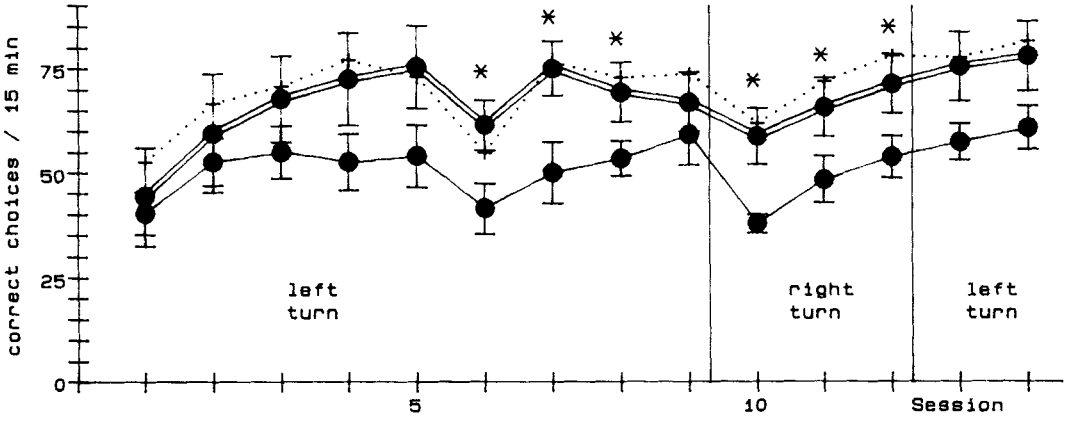

FIG.2b Learning performance of sham-operated (+—+) and NBM-lesioned rats with (●═══●) and without (●——●) KFM 19 pretreatment. Asterisks indicate significant differences ($p<0.05$) between mean values of treated and nontreated groups at single training sessions.

acquisition period the differences between treated and non-treated animals were particularly pronounced.

In the second experimental series the NBM lesioned rats turned out to be markedly impaired in their learning ability. Again, lesioned rats pretreated with KFM 19 showed higher rates of correct choices than the lesioned but nontreated group. This was true in particular during periods of reacquisition following a general decay in performance (due to weekend training deficit, or due to reversal of the experimental task). Thus, KFM 19 treatment significantly improved learning performance in old aged and NBM-lesioned rats (cf. FIG. 2b).

Discussion

Starting with the hypothesis that selective adenosine A_1 antagonists may be useful for treatment of cognitive deficits in humans, the novel compound KFM 19 was tested in a variety of biochemical and pharmacological models. Besides receptor binding assays, the test models applied can be divided into mechanism-related and symptom-related tests performed in vitro as well as in vivo. In the receptor binding studies, KFM 19 showed high affinity to and selectivity for adenosine A_1 receptors. These data compare to those of dipropyl-CPX which is among the most potent and selective A_1 antagonists known so far. The mechanism-related tests demonstrated KFM 19 to potently antagonise adenosine or adenosine agonist induced effects in vitro and in vivo. The enhancing effects on acetylcholine release and synaptic transmission in rat hippocampal slices are such as would be expected for an adenosine antagonist. However, in contrast to caffeine which is only a weak adenosine antagonist, KFM 19 does not exert major excitatory effects when given alone: locomotor activity in mice is mildly stimulated only in high doses whereas the ED_{50} value for antagonism of DHPA induced locomotor depression is much lower; EEG arousal and sleep disorders are not elicited in normal animals in a dose range where a functional antagonism versus scopolamine is already present.

Moreover, it can be concluded from these experiments that KFM 19 has good bioavailability following parenteral as well as oraladministration. Finally, improvement of learning performance in old-aged and NBM-lesioned rats by KFM 19 supports the hypothesis that

A_1 antagonists such as KFM 19 can have a therapeutic potential for treatment of cognitive deficits. The main reason why this has not been persued so far seems to be the very low aqueous solubility and poor bioavailability of selective A_1 antagonists presently available. KFM 19 offers the opportunity to further investigate CNS effects and therapeutic potential of A_1 antagonists in in vivo models.

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