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Adenosine mechanisms are not affected by antidepressant concentrations of desipramine

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We have evaluated the proposal that adenosine may mediate some of the effects of tricyclic antidepressant therapy. In-vitro desipramine (DMI) (1–10 μM) did not affect adenosine or 2-chloroadenosine-induced inhibition of lipolysis or the adenosine stimulated formation of cyclic (c)AMP in the hippocampal slice. However, very high concentrations of desipramine (0·2–0·5 mM) as well as some detergents potentiated the stimulatory effect of adenosine on cAMP formation. The ATP, ADP and AMP contents in slices were unaffected as was the electrically evoked release of purines. Long-term treatment in-vivo with antidepressants in clinically relevant doses did not alter the sensitivity of adenosine receptor mediated cAMP formation in-vitro while the β-adrenoceptor-mediated formation was depressed by desipramine or imipramine treatment but not by zimelidine or fluoxetine treatment. It is concluded that actions on central adenosine mechanisms are unlikely to play any important role in the therapeutic effects of tricyclic antidepressants.

It has been proposed that adenosine may contribute to the effects of antidepressants (see Stone 1981; Phillis & Wu 1981, 1982). In 1971 Kodama et al found that desipramine increased cAMP levels in guinea-pig cortical tissues by a theophylline sensitive mechanism. Since theophylline acts as a rather selective inhibitor of adenosine actions in the central nervous system (cns) (cf Fredholm 1980) these results could indicate that adenosine mediates certain actions of desipramine. These results were confirmed and extended to other antidepressants (Sattin et al 1978), and it was shown that iontophoretically applied antidepressants potentiated actions of adenosine on rat cortical neuron firing (Stone & Taylor 1979). One possible mechanism behind the effect could be inhibition of adenosine inactivation. Phillis & Wu (1981) have reported that in-vitro desipramine inhibits adenosine uptake by 20% at a concentration of 18 μM, which confirms that desipramine has the potential of interacting with adenosine mechanisms.

Optimal plasma concentration of desipramine is well below 1 μM (Risch et al 1981). Even though tissue levels are considerably higher at steady state (Risch et al 1981) there is evidence that this represents an inactive fraction (Borgå et al 1970). In the present study we have therefore chosen to focus on concentrations not higher than 10 μM desipramine. Even though this concentration is likely to exceed the free level necessary by many orders of magnitude (Peroutka & Snyder 1981) we have not been able to find any consistent effects on the evoked release or action of adenosine suggesting that interaction with adenosine is unimportant as a mechanism for the antidepressant effect of desipramine.

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Methods

All experiments were carried out with male Sprague-Dawley rats (Anticimex strain) 180–280 g given free access to food and water.

Long term treatment. All drugs were given orally twice daily in clinically relevant doses (2 × 5–20 pmol kg^{-1} day^{-1}). The treatment was given for 2 weeks and after a drug-free period of approximately 24 h the rats were killed. For further details see legends to figures.

cAMP accumulation in hippocampal slices. These experiments were carried out as decribed by Fredholm et al (1982). Slices were labelled for 15 min with 1·5 μCi [^{3}H]adenine/slice. The slices were washed and transferred to vials containing a Krebs-Ringer bicarbonate buffer (KRB) and additions and was kept at 37 °C for 15 min. The [^{3}H]cAMP was separated from other labelled purines (mainly [^{3}H]ATP) by combined alumina and Dowex 50 chromatography. The results were expressed as percent of the total amount of label taken up that was converted to labelled cAMP. In all these experiments a non-xanthine cAMP phosphodiesterase inhibitor Rolipram (30 μM) was present.

Electrical field stimulation of hypothalamic slices. Slices were labelled with [^{14}C]adenine and [^{3}H]noradrenaline. The slices were continuously superfused with KRB and 5 min fractions were collected. Biphasic rectangular pulses of 20 V with 1 ms duration were given with a frequency of 10 Hz for 5 min. The fractional release rate of tritium and carbon 14 was calculated and expressed as percent min^{-1} of the total content (for details see Fredholm & Jonzon 1981).

Nucleotide content in slices. Slices from hippocampus or hypothalamus were incubated for 45 min (hippocampus) or 3 h (hypothalamus) at 37 °C in KRB gassed with 5% CO_2 in oxygen in the absence or presence of 10 μM desipramine. The slices were homogenized in ice-cold 1 M perchloric acid and centrifuged (2600 rev min^{-1} for 15 min at 4 °C, MSE Coolspin). The protein content was determined. The supernatant was neutralized with KOH and buffered with Tris. ATP, ADP and AMP were determined by reversed phase hplc essentially as described by Schweinsberg & Loo (1980). The energy charge was calculated as the ratio (ATP + ADP/2)/(ATP + ADP + AMP) in the slice.

Lipolysis in-vitro. Fat cells were isolated from epididymal fat pads essentially as described by Rodbell (1964).

Cut pieces of the tissue were gently shaken for 30–60 min at 37 °C in a Krebs-Ringer Phosphate buffer (KRP) containing 30 g BSA litre^{-1} and collagenase 3 g litre^{-1} and thereafter passed through a nylon mesh. The filtrate was washed with KRP-BSA and then briefly centrifuged (200 rev min^{-1}). This washing procedure was repeated twice. The cell suspension was then diluted to 250 000–600 000 cells ml^{-1} with KRP-BSA. Aliquots of 50 µl were incubated at 37 °C for 10 min in 2 ml KRP-BSA with additions. The reaction was terminated with $ZnSO_4$ and thereafter $Ba(OH)_2$ and the glycerol content was determined with fluorometric technique.

Chemicals. [^{3}H]Adenine, 25 Ci mmol^{-1}, was obtained from Radiochemical Centre, Amersham, UK, [^{14}C]-adenine, 55·6 mCi mmol^{-1} and [^{3}H]noradrenaline ([^{3}H]-NA), 4·3 Ci mmol^{-1} were obtained from New England Nuclear, Boston, MA, USA. Desipramine, imipramine, and phentolamine were obtained from CIBA-GEIGY, Basel. Fluoxetine was obtained from Duphar B.V., Weesp, The Netherlands. Byk-Gulden Pharmazeutika, Konstanz, BRD kindly provided us with 5′-*N*-ethylcarboxamidoadenosine (NECA). Rolipram (ZK 62·711) was from Schering AG, Berlin. Theophylline was obtained from ACO, Sweden as the ethylendiamine salt and zimelidine from Astra, Sweden.

Table 1. Effect of desipramine on 2-chloroadenosine-induced inhibition of lipolysis (glycerol release) in rat fat cells. The cells (57 000 ml^{-1}) were preincubated with or without desipramine (DMI, 1 or 10 µM) and 2-chloroadenosine (10^{-9}–10^{-6} M) for 15 min. Lipolysis was stimulated with NA (10^{-8} M) and adenosine deaminase (ADA 1 mg ml^{-1}). Confidence intervals (95%) of Hill coefficients and log EC50 values (in nM) of triplicate determinations.

	log (EC50 nM)	Hill coefficient
Control	1·37–1·65	0·56–1·00
1 µM DMI	1·26–1·58	0·21–1·33
10 µM DMI	1·10–1·28	0·78–1·14

Results and discussion

Desipramine at 1 and 10 µM only marginally affected 2-chloroadenosine-induced inhibition of lipolysis in fat cells (Table 1). Desipramine (10 µM) did not affect the adenosine-induced inhibition of theophylline (1 mM) stimulated lipolysis (not shown). Since 2-chloroadenosine inhibits via an action on A1 adenosine receptors (see Fredholm & Lindgren 1984), these findings suggest that desipramine does not potentiate adenosine effects at A1 receptors.

Neither DMI nor zimelidine in concentrations up to 10 µM affected adenosine-induced stimulation of [^{3}H]cAMP accumulation in rat hippocampal slices (Table 2). Concentrations of desipramine above 0·1 mM potentiated the effect of adenosine (Table 2, lower panel). Thus we could confirm previous findings that high concentrations of tricyclic antidepressants can raise

Table 2. Lack of effect of DMI and zimelidine on [^{3}H]cAMP accumulation in rat hippocampal slices (upper panel) and the potentiating effect of very high concentrations of desipramine and other detergents (sodium lauryl sulphate, (SDS), and Triton X100) on [^{3}H]cAMP accumulation in rat hippocampal slices (lower panel). Mean, s.d. and number of determinations (n).

		[^{3}H]cAMP (% of total ^{3}H) Mean	s.d.	(n)
Rolipram 30 µM		0·24	0·08	9
+Adenosine	10^{-5} M	0·58	0·16	15
+desipramine	10^{-7} M	0·52	0·06	3
	10^{-6} M	0·46	0·10	6
	3×10^{-6} M	0·60	0·12	3
	10^{-5} M	0·60	0·20	12
+zimelidine	10^{-6} M	0·54	0·02	3
	10^{-5} M	0·50	0·04	3
Rolipram 30 µM		0·28	0·04	3
+Adenosine	10^{-5} M	0·72	0·17	3
+desipramine	2×10^{-4} M	0·84	0·24	3
+desipramine	5×10^{-4} M	2·85	0·43	3
+SDS	0·2%	1·36	0·31	3
+Triton X100	0·2%	0·88	0·08	3
+Triton X100	1·0%	0·88	0·21	3

cAMP accumulation, but suggest that this effect may be at least partly due to non-specific actions, since it was shared by two detergents. Adenosine stimulates cAMP accumulation in rat hippocampus by actions on adenosine receptors of the A2-subtype (Fredholm et al 1982). Thus, even at concentrations 20 times higher than the therapeutic levels (10 vs 0·5 µM (Risch et al 1981)) desipramine does not potentiate adenosine action on A2-receptors. Furthermore, the effect of adenosine in rat hippocampal slices is markedly enhanced by drugs that impair adenosine uptake (Fredholm et al 1982). Thus, the present results suggest that even at 10 µM desipramine does not cause a functionally important inhibition of adenosine uptake.

Since the acute and chronic effects of the antidepressant drugs may differ (Mobley & Sulser 1981), we investigated the effect of long-term treatment with several antidepressants on [^{3}H]cAMP accumulation in these slices. The results are given in Table 3. In

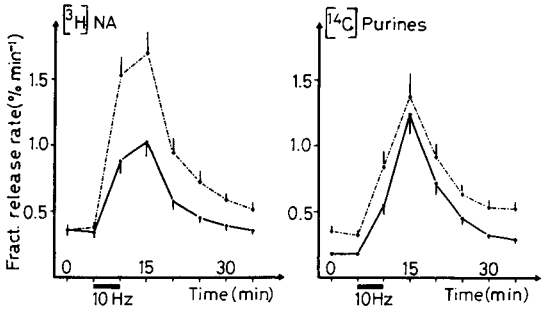

FIG. 1. The effect of desipramine (◆–·–◆) on the fractional release of tritium and carbon 14 after [^{3}H]noradrenaline and [^{14}C]adenine labelling from rat hypothalamic slices. The slices were stimulated (20 V, 2 ms, 10 Hz) for 5 min (indicated with bar). Mean and s.e.m. of 28 (desipramine) and 32 (control) (●——●) experiments.

Table 3. Effect of long term treatment with antidepressant drugs on noradrenaline and NECA-induced accumulation of [^{3}H]cAMP in slices of rat hippocampus. Mean ± s.e.m. Number of determinations within parentheses.

Treatment	[^{3}H]cAMP accumulation (% of total ^{3}H) No drug	NA 30 μM +phent. 10 μM	NA 30 μM +propr. 10 μM	NECA 1 μM
Saline	0·27 ± 0·01 (33)	1·88 ± 0·13 (33)	0·80 ± 0·06 (33)	1·28 ± 0·09 (33)
Desipramine 2 × 5–20 μmol kg^{-1} day^{-1}	0·34 ± 0·04 (9)	1·44 ± 0·17* (9)	0·75 ± 0·09 (9)	1·37 ± 0·18 (9)
Zimelidine				
2 × 5 μmol kg^{-1} day^{-1}	0·27 ± 0·03 (9)	1·86 ± 0 26 (9)	0·80 ± 0·10 (9)	0·94 ± 0·10* (9)
2 × 10 μmol kg^{-1} dy^{-1}	0·24 ± 0·01 (12)	1·90 ± 0·16 (12)	0·80 ± 0·04 (12)	1·22 ± 0·10 (12)
2 × 20 μmol kg^{-1} day^{-1}	0·26 ± 0·01 (9)	1·84 ± 0·20 (9)	0·68 ± 0·07* (8)	1·21 ± 0·10 (8)
Imipramine 2 × 20 μmol kg^{-1} day^{-1}	0·27 ± 0·02 (4)	1·14 ± 0·08* (4)	0·64 ± 0·12 (4)	1·20 ± 0·18 (4)
Fluoxetine 2 × 10 μmol kg^{-1} day^{-1}	0·25 ± 0·02 (3)	1·59 ± 0·12 (3)	0·76 ± 0·15 (3)	1·19 ± 0·08 (3)

(* = significantly different from saline treated controls, $P < 0.05$, Student's t-test.)

agreement with previous results imipramine and DMI treatment caused an inhibited β-adrenoceptor mediated response. The two atypical antidepressants, zimelidine and fluoxetine did not have this effect, which is also in agreement with previous results (Mobley & Sulser 1981). There were no consistent effects on NECA induced accumulation of [^{3}H]cAMP, suggesting that adenosine receptors are not regulated by antidepressants.

The results presented in Fig. 1 demonstrate the expected enhancement of [^{3}H]NA overflow from stimulated hypothalamic slices. By contrast there were no changes in the release of [^{14}C]adenine metabolites evoked by field stimulation. There was a tendency that basal release of [^{14}C]adenine- derived metabolites was enhanced by DMI. However, the energy charge in slices from hippocampus (0·83 ± 0·01) and hypothalamus (0·84 ± 0·01) was unaffected by DMI treatment (0·84 ± 0·01 and 0·83 ± 0·01 respectively, mean and s.e.m. of 3–6 determinations). Furthermore the purine nucleotide content was not altered by 10 μM DMI treatment

Thus, the effect of tricyclic antidepressants on formation, inactivation and effects of adenosine are likely to be small to insignificant. It is suggested that the reported effects of antidepressant drugs (see introduction) are pharmacologically irrelevant and that they may depend on for example the chaotropic actions of concentrated (above 0·1 mM) solutions of antidepressant drugs.

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