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EFFECTS OF DIAZEPAM ON ADENOSINE AND ACETYLCHOLINE RELEASE FROM RAT CEREBRAL CORTEX: FURTHER EVIDENCE FOR A PURINERGIC MECHANISM IN ACTION OF DIAZEPAM

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1 Diazepam administered intraperitoneally (0.25 mg/kg) enhanced the rate of efflux of [^{3}H]-adenosine and its metabolites from rat cerebral cortex. At a lower dose (0.05 mg/kg), this effect could be detected in only one of four rats.

2 Diazepam (0.05 and 0.25 mg/kg i.p.) depressed acetylcholine release from the rat cerebral cortex. Its effect was reversed by theophylline.

3 Theophylline (15 and 30 mg/kg) enhanced acetylcholine release from the rat cerebral cortex. Diazepam (0.25 mg/kg) administered after theophylline failed to cause a reduction in the rate of release, rather there appeared to be a further enhancement of release.

4 Pentobarbitone sodium (5, 10 and 15 mg/kg i.p.) did not elicit any increase in adenosine release.

5 These results support the proposal that benzodiazepines may exert their pharmacological actions by preventing adenosine uptake, thus enhancing the levels of extracellular adenosine.

Introduction

Although potentiation of the actions of γ-aminobutyric acid (Zakusov, Ostrovskaya, Kozhechkin, Marovich, Molodavkin & Voronina, 1977; Karobath & Sperk, 1979; Costa & Guidotti, 1979) is believed to be responsible for some of the primary central actions of the benzodiazepines, it has also been suggested that benzodiazepines may act by potentiating the effects of adenosine, a proposed intercellular mediator in the central nervous system (Phillis, 1979).

Adenosine has a powerful depressant action on the spontaneous activity of neurones in many regions of the brain (Phillis, Kostopoulos & Limacher, 1974; Phillis & Kostopoulos, 1975; Kostopoulos, Limacher & Phillis, 1975; Kostopoulos & Phillis, 1977) and its release from the cerebral cortex is enhanced by electrical stimulation (Sulakhe & Phillis, 1975). Diazepam is a potent inhibitor of the uptake of adenosine by brain slices and cultured glial cells (Mah & Daly, 1976; Hertz, Wu & Phillis, 1979) and it potentiates the depressant actions of iontophoretically applied adenosine on rat cerebral cortical neurones (Phillis, 1979). The depressant effects of flurazepam, a related benzodiazepine, on rat cortical neurones are blocked by the adenosine antagonist, theophylline (Phillis, Edstrom, Ellis & Kirkpatrick, 1979a), implying either that flurazepam has a direct action on the adenosine receptor or that enhancement of the levels or actions of endogenously released adenosine causes its depressant effects.

Interest in the interactions between benzodiazepines and purines has been intensified by recent reports that various purines, including adenosine, inosine and hypoxanthine, can compete for the benzodiazepine-binding site in brain tissue (Skolnick, Marangos, Goodwin, Edwards & Paul, 1978; Marangos, Paul, Parma, Goodwin, Syapin & Skolnick, 1979; Asano & Spector, 1979; Damm, Muller & Wollert, 1979). These studies have been variously interpreted as suggesting that benzodiazepines bind to a purine receptor (Skolnick *et al.*, 1978; Marangos *et al.*, 1979) or to an adenosine transport site (Hertz *et al.*, 1979).

To obtain *in vivo* evidence for an action of diazepam at the adenosine transport site, the effect of clinically used doses of this benzodiazepine on adenosine release from the rat cerebral cortex has been ascertained. Furthermore, since it is known that adenosine depresses acetylcholine (ACh) release from the cerebral cortex (Jhamandas & Sawynok, 1976), we have studied the effect of the same doses of diazepam on the release of ACh from the cerebral cortex. Our findings indicate that diazepam enhances adenosine release, depresses ACh release and that the latter effect can be blocked by theophylline.

Methods

Experiments were performed on 34 adult male Sprague-Dawley or Wistar rats (285 to 660 g). Anaesthesia was induced with halothane and the trachea cannulated. The animals were then placed in a stereo-

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taxic head holder (Narishige) and anaesthesia was maintained with a mixture of nitrous oxide (75%), oxygen (25%) and methoxyflurane. After completion of the surgical procedures, the methoxyflurane vaporizer was adjusted to ensure that the animals would be adequately anaesthetized and no further changes were then made to the flow regulators in those experiments in which diazepam was administered. When the effects of pentobarbitone sodium on adenosine release were being ascertained, it was sometimes necessary to reduce the methoxyflurane flow. Body temperature was kept constant at 37°C by an electric heating pad controlled by a feedback circuit using a rectal probe.

Adenosine release

Both cortical hemispheres were exposed, leaving a thin crest of bone along the midline. The dura was removed and rectangular cups with inside dimensions of 5 × 8 mm were placed bilaterally on the surface of the cortex. Leakage from the cups was prevented by a coating of silicone grease on the cup surface in contact with the brain. When the cups were in place, the exposed cortical, bone and muscle surfaces were covered with a layer of 4% agar in physiological saline or with sterile, pyrogen-free physiological saline (Ringer's Injection, Abbott) placed within the two cups. The cortical surface within each cup was then incubated for 45 min with 100μl solutions of [2,8-^{3}H]-adenosine (0.1mM; specific activity of 0.1 Ci/mol) in saline.

In six experiments the cup over one cortex was filled with [2,8-^{3}H]-adenosine (0.1 mM, specific activity of 0.1 Ci/mol) and that over the other cortex with [^{14}C]-urea (1.6 mM, specific activity 10 Ci/mol) during the incubation period.

The cups were then rinsed ten times in rapid succession with warmed physiological saline and subsequently refilled with 100 μl of saline. Thereafter the contents of the cups were withdrawn every 15 min and replaced with fresh saline. The samples were mixed with 5 ml of PCS scintillation fluid (Amersham Corporation) and counted in a Nuclear Chicago Isocap 300 liquid scintillation counter.

Twelve rats were injected intraperitoneally with diazepam (0.05 or 0.25 mg/kg). Eight rats received intraperitoneal injections of pentobarbitone sodium (5, 10 and 15 mg/kg) at 45 min intervals and three animals were given a single injection of 15 mg/kg pentobarbitone sodium.

Acetylcholine release

Eleven rats were used in these experiments. Bone overlying the cerebral hemispheres was removed, including that in the mid-line and a single Perspex cylinder (cup) with an inside diameter of 9 mm was placed on the dorsal surface of the brain, covering both exposed hemispheres. Exposed surfaces around the cup were covered with 4% agar in physiological saline and the cup was filled with a solution of 50 μg/ml neostigmine bromide in physiological saline. This solution was changed three times at 20 min intervals and the cup was then rinsed several times with saline and then filled with 0.4 ml of the neostigmine-containing solution. After 15 min the solution in the cup was collected and replaced with fresh solution. Subsequent collections were made at 15 min intervals. The ACh content of each cortical perfusate was determined by bioassay on the hearts of the bivalve mollusc, *Mercenaria mercenaria* (Jhamandas, Phillis & Pinsky, 1971). At the end of each experiment the hearts were perfused with benzoquinonium chloride (5×10^{-7} M) (Mytolon, an ACh-antagonist)-containing sea water and in every instance the inhibitory effect of the cortical perfusates was abolished.

Results

Adenosine release

The release of labelled adenosine (and its metabolites) from 39 cerebral hemispheres was studied. The amount of labelled material released showed an exponential decline which tended to reach a plateau phase 2 h after the end of incubation. Intraperitoneal drug injections were therefore given 120 min after the start of sample collection (i.e. after removal of the eighth sample).

Diazepam Figure 1 illustrates an experiment in which 0.25 mg/kg of diazepam was administered to the animal. The rate of ^{3}H-efflux declined rapidly from an initial high value and reached a fairly stable level some 75 min after the start of sample collection. Diazepam was injected intraperitoneally at 120 min and evoked a marked increase in the rate of ^{3}H-efflux which was apparent in the next three cortical perfusates. The rate of release of ^{3}H then returned to pre-diazepam levels.

The results of six such experiments showing the effect of diazepam (0.25 mg/kg) on [^{3}H]-adenosine release from rat cerebral cortex are presented in Table 1. The mean rate of ^{3}H-efflux during the 7th and 8th samples (those immediately preceding the administration of diazepam) represents the control rate release (100%). During the 30 min period immediately following the administration of diazepam, the rate of efflux of [^{3}H]-adenosine was increased in 9 cortices of 6 rats. The mean percentage increase above control level in these animals was 99.9 ± 49 (s.e. mean). During the subsequent 30 min period the mean rate of release from these animals was increased above con-

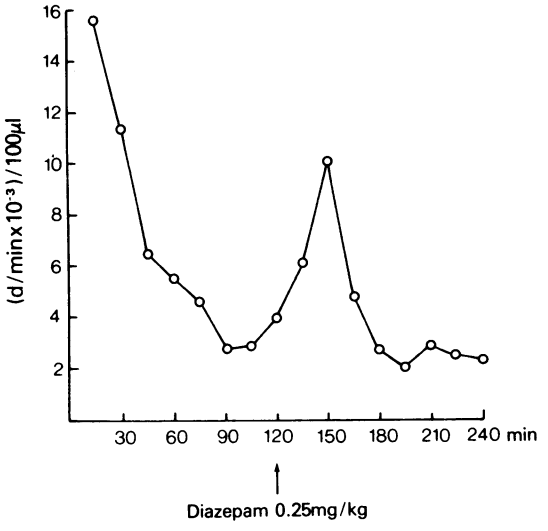

Figure 1 Efflux of labelled adenosine from the rat cerebral cortex. After preincubation with [^{3}H]-adenosine for 45 min, the cortex was rinsed ten times and the cup was refilled with 100 µl of physiological saline. Efflux studies were then carried out for 4 h. The cup contents were removed for counting every 15 min and replaced with fresh saline. After 2 h (at the point indicated by the arrow), diazepam (0.25 mg/kg) was injected intraperitoneally. Collections were continued for a further 2 h.

trol levels in only 6 instances (mean percentage increase, 14.9 ± 6.3). With one exception, no increases were observed during collections made more than 60 min after the injection of diazepam. In two animals (4 cortices) no increase in ^{3}H-efflux was observed following diazepam (0.25 mg/kg) administration. Since the diazepam may not have been absorbed at the usual rate in these two animals, these data are not included in Table 1.

In three experiments [^{14}C]-urea replaced [^{3}H]-adenosine in one of the cups and the effects of diazepam on adenosine and urea release could then be compared. The results of one of these experiments are shown in Figure 2. The decline in urea (as ^{14}C-d/min) release paralleled the fall in adenosine (as ^{3}H-d/min) efflux, but unlike [^{3}H]-adenosine, the release of [^{14}C]-urea was not affected by diazepam. The rate of urea release was also unaffected by diazepam in the other two experiments.

Diazepam (0.05 mg/kg) was administered to 4 rats and its effects on ^{3}H-efflux were recorded from 6 cortices. In only one instance was there an increase in adenosine release, and this occurred only from one hemisphere. This dose level therefore appears to be near the threshold for eliciting a detectable increase in [^{3}H]-adenosine efflux.

Pentobarbitone sodium Pentobarbitone sodium was administered intraperitoneally in doses of 15 mg/kg (3 rats; 5 cortices) or in an ascending sequence of doses of 5, 10 and 15 mg/kg at 45 min intervals (8 rats; 13 cortices). In these amounts pentobarbitone did not increase ^{3}H-efflux from the cerebral cortex. There was an indication in some experiments that pentobarbitone may actually have depressed [^{3}H]-adenosine release, but the continual decline in release typical of labelled isotope efflux studies such as these, makes it impossible to determine the significance of these observations. The results of one experiment are illustrated in Figure 3. Pentobarbitone (5, 10 and 15 mg/kg) failed to increase the rate of ^{3}H-efflux from either cortical hemisphere. Similar results were obtained with the other 10 animals.

Acetylcholine release

Spontaneous release In rats maintained under light nitrous oxide and methoxyflurane anaesthesia, the rate of spontaneous release of ACh into a cup overlying both cortical hemispheres was relatively uniform in any single animal, but varied somewhat from animal to animal. The mean rates of release recorded during three successive 15 min collection periods from 11 animals were 405 ± 77.6 (s.e. mean) pg min^{-1}

Table 1 Effects of diazepam on the efflux of [^{3}H]-adenosine and its metabolites from the rat cerebral cortex[1]

	% *increase in release post diazepam* (*0.25 mg/kg*)		
Rat. No.	0–30 min	30–60 min	60–90 min
1	24	20	0
	3	0	0
2	131	6	0
	245	42	0
3	7	0	0
	0	6	0
4	36	14	0
	495	61	2
5	19	0	0
6	39	0	0
Means ± s.e.	99.9 ± 49 (P = 0.05)[2]	14.9 ± 6.3 (0.05 > P > 0.01)	0.2

[1] Control release (100%) is the average of ^{3}H-efflux rates (in d/min) during the two 15 min periods immediately preceding diazepam administration. Post diazepam release rates are derived from the averages of pairs of successive 15 min collection periods during three consecutive 30 min periods.

[2] Significance levels calculated by the Student's t test.

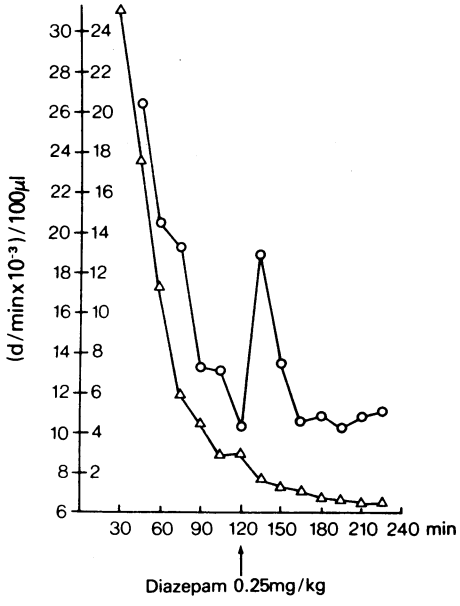

Figure 2 Efflux of ^{3}H-labelled adenosine (O) and ^{14}C-labelled urea (△) from the left and right cortical hemispheres of a rat brain, respectively. During the preincubation period the left cup contained labelled adenosine and the right cortical cup labelled urea. After 45 min both cups were rinsed thoroughly and collections were made at 15 min intervals as described in the legend for Figure 1. After 2 h diazepam (0.25 mg/kg) was injected intraperitoneally. Collections were continued at 15 min intervals for a further 105 min.

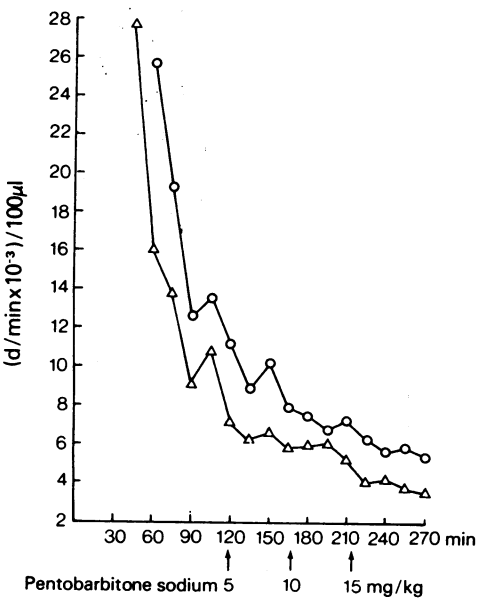

Figure 3 An experiment showing the lack of effect of pentobarbitone sodium (5, 10 and 15 mg/kg) on [^{3}H]-adenosine release from the left (O) and right (△) cortical hemispheres of a rat. Both hemispheres were incubated with labelled adenosine. Pentobarbitone sodium was administered intraperitoneally 120, 165 and 210 min after the start of sampling.

cm^{-2}, 468 ± 72.1 pg min^{-1} cm^{-2} and 448 ± 75.5 pg min^{-1} cm^{-2}.

Diazepam Diazepam decreased the resting output of ACh. Table 2 shows the decreases from control release levels during the first and second 30 min periods of collection following the injection of diazepam (0.05 and then 0.25 mg/kg). Significant reductions in the release of ACh were observed with both doses of diazepam. Release was depressed for periods of 60 to 90 min, after which recovery to control levels occurred.

A typical experiment is shown in Figure 4. Diazepam (0.05 mg/kg) decreased the output of ACh from 530 pg min^{-1} cm^{-2} to 318 pg min^{-1} cm^{-2} and after a second injection of diazepam (0.25 mg/kg) the rate of release further decreased to 80 pg min^{-1} cm^{-2}. Theophylline (30 mg/kg) administered intraperitoneally caused an immediate reversal of the depression and release increased to 743 pg min^{-1} cm^{-2}.

Theophylline (15 mg/kg, 2 animals; 30 mg/kg, 2 animals) reversed the depressant effects of diazepam on ACh release in all 4 animals tested and as shown in Table 2, enhanced the rate of ACh release in comparison with the control rates.

Theophylline In five experiments theophylline (15 mg/kg) was administered before diazepam and in each instance the rate of ACh release was increased. Two animals, in which the increase in ACh release was not particularly pronounced were given a further injection of theophylline (15 mg/kg), which elicited a more substantial increase in release. Diazepam (0.25 mg/kg), administered to all five animals 60 min after the last theophylline injection, failed to decrease ACh release; rather there was a further increase in release which became statistically significant during the 30 to 60 min collection periods.

One of these experiments is illustrated in Figure 5. Theophylline (15 mg/kg) increased the rate of ACh release from 265 pg min^{-1} cm^{-2} to a maximum of 849 pg min^{-1} cm^{-2} in the second post-injection collection period. The release rate then fell slightly to 637 pg min^{-1} cm^{-2} in the fourth post-injection collection period. Diazepam (0.25 mg/kg) evoked a further enhancement of release which rose to 1274 pg min^{-1}

Table 2 Effects of diazepam and theophylline on cerebral cortical acetylcholine (ACh) release (pg min^{-1} cm^{-2})[5]

Rat No.	Control[1] rate of release	*Diazepam (0.05 mg/kg)* Mean rate of release during first 30 min	*Diazepam (0.05 mg/kg)* Mean rate of release during second 30 min	*Diazepam (0.25 mg/kg)* Mean rate of release during first 30 min	*Diazepam (0.25 mg/kg)* Mean rate of release during second 30 min	*Theophylline (15 or 30 mg/kg)* Mean rate of release during first 30 min	*Theophylline (15 or 30 mg/kg)* Mean rate of release during second 30 min
1	1061	1008	371	318	318		
2	318	318	201	212	212		
3	531	344	291	185	106	902	1061
4	531	371	318	265	93	689	743
5	743	690	478	425	371	637	1433
6	318	159	159	159	96	478	531
Means ± s.e.	584 ± 105	482 ± 116 (NS)[2]	303 ± 43 ($0.05 > P > 0.01$)	260 ± 37 ($0.05 > P > 0.01$)	199 ± 45 ($0.01 > P > 0.001$)	676 ± 76[4] (NS)	942 ± 170[4] (NS)

		Theophylline (15 mg/kg) Mean rate of release during first 30 min	*Theophylline (15 mg/kg)* Mean rate of release during second 30 min	*Theophylline (15 mg/kg)* Mean rate of release during first 30 min	*Theophylline (15 mg/kg)* Mean rate of release during second 30 min	*Diazepam (0.25 mg/kg)* Mean rate of release during first 30 min	*Diazepam (0.25 mg/kg)* Mean rate of release during second 30 min
7	425			902	955	796	743
8	159	159	185	159	531	1432	1751
9	265			743	690	690	1168
10	372			531	796	902	1061
11	318	557	318	372	425	425	425
Means ± s.e.	307 ± 41			541 ± 117 ($0.1 > P > 0.05$)	679 ± 84 ($0.01 > P > 0.001$)	849 ± 148[3] ($0.01 > P > 0.001$)	1029 ± 199[3] ($0.01 > P > 0.001$)

[1] Control release represents average of release during the two 15 min periods immediately preceding diazepam or theophylline administration.
[2] Significance levels calculated by the Student's *t* test. Each value is compared to the original control rate of release.
[3] These values are highly significantly different from the mean rate of release immediately before theophylline administration ($P < 0.001$ and $0.001 < P < 0.01$ respectively).
[4] These values are not significantly different (NS) from the immediately pre-diazepam release rates.
[5] Eleven rats were used in these experiments. In rats Nos 1 to 6, once ACh efflux rates had stabilized, diazepam (0.05 mg/kg) was injected intraperitoneally and release rates were mesured during four successive 15 min periods. A second injection of diazepam (0.25 mg/kg) was then administered and efflux rates were measured for 60 min. Rats Nos 3 to 6 were then given 15 (2 rats) or 30 (2 rats) mg/kg theophylline intraperitoneally and ACh collections were continued for at least another hour.
Rats Nos 7 to 11 received theophylline first. ACh release in two rats (Nos 8, 11) did not respond to the initial dose of 15 mg/kg and these animals received a further 15 mg/kg. Rats Nos 7, 9 and 10 received only one dose of 15 mg/kg. Sixty minutes after the final injections of theophylline, diazepam (0.25 mg/kg) was given to all animals and collections were continued for a further 60 min or longer.

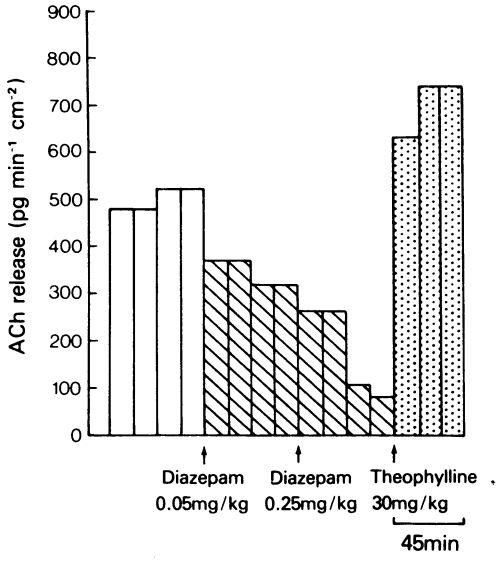

Figure 4 Rates of acetylcholine (ACh) release from the rat cerebral cortex (the cup covered a portion of both hemispheres) before and after intraperitoneal injections of diazepam (0.05 and 0.25 mg/kg) and theophylline (30 mg/kg). Each collection period was of 15 min duration.

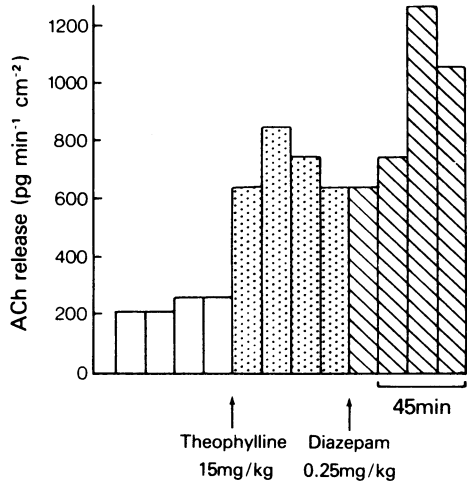

Figure 5 Rates of acetylcholine (ACh) release from the rat cortex before and after intraperitoneal injections of theophylline (15 mg/kg) and diazepam (0.25 mg/kg). Each collection period was of 15 min duration.

cm^{-2} in the third post-injection collection period. Release then started to decrease, falling to 1062 pg min^{-1} cm^{-2} in the fourth collection period and at this point the experiment was terminated.

Discussion

The experiments described in this paper show that diazepam affects the release of acetylcholine and adenosine or its derivatives from the rat cerebral cortex *in vivo*. Endogenous ACh release was measured by a sensitive and reliable bioassay technique (the *Mercenaria mercenaria* heart). Adenosine release was measured by prelabelling the endogenous pools with [^{3}H]-adenosine. Following such prelabelling, most of the radioactive marker is associated with adenine nucleotides and adenosine, and only a small proportion of the adenosine is metabolized to inosine, hypoxanthine and adenine (Kuroda & McIlwain, 1974). After release, the radioactive label output consists mainly of adenosine and its metabolites, inosine and hypoxanthine, with a small amount of labelled nucleotides (Kuroda & McIlwain, 1974; Sulakhe & Phillis, 1975; Lewin & Bleck, 1976).

Diazepam was administered intraperitoneally in doses of 0.05 and 0.25 mg/kg. The latter dose closely approximates the therapeutic dose used by oral or intramuscular administration in man (5 to 15 mg). The intraperitoneal route of administration was selected for the present experiments in preference to intravenous injection because the rate of absorption following the former method of administration should simulate absorption after oral or intramuscular injection more closely. Intravenous administration is followed initially by very high serum levels of diazepam in comparison with those observed after oral or intramuscular administration in man (Hillestad, Hansen, Melsom & Drivenes, 1974). A possible disadvantage of the intraperitoneal route of administration is the variability in absorption rates with this technique. Thus poor absorption may be the explanation for the lack of effect of diazepam in two of the present experiments.

Diazepam increased the rate of efflux of adenosine and its metabolites from the *in situ* rat cerebral cortex. Urea release, used as an indicator of non-specific alterations in release, was unaffected, signifying that the alterations in adenosine release reflect a selective effect of this benzodiazepine. The effect is unlikely to have been a consequence of an increase in the depth of anaesthesia and/or subsequent fall in oxygen tension in the brain as no increase in adenosine release was observed after administration of the barbiturate anaesthetic, pentobarbitone sodium, which both deepened anaesthesia and depressed respiration. Our results extend an earlier observation (Lewin & Bleck, 1976) that another barbiturate, phenobarbitone, has no effect on adenosine release from brain slices.

The adenosine-release enhancing effect of diazepam is probably related to inhibition of adenosine uptake (Mah & Daly, 1976; Hertz *et al.*, 1979), and is consistent both with the potentiating action of this agent on adenosine-elicited depression of neuronal firing (Phillis, 1979; Phillis, Kostopoulos, Edstrom & Ellis, 1979c), and the antagonism of flurazepam-evoked depression of neuronal firing by the adenosine antagonist, theophylline (Phillis *et al.*, 1979a).

Adenosine, applied topically, depresses ACh release from the guinea-pig cerebral cortex and this effect is antagonized by theophylline (Jhamandas & Sawynok, 1976). Adenosine also depresses ACh release at peripheral cholinergic nerve terminals by a theophylline-sensitive mechanism (Ribeiro, 1978; Ribeiro, Dominguez & Goncalves, 1979). The depressant action of diazepam on rat cerebral cortical ACh release observed in the present study may therefore be a consequence of its enhancement of extracellular adenosine levels. The suggestion that a purine receptor is involved receives further support from the observation that theophylline, administered either before or after diazepam, abolishes or reverses the depressant effect of the latter on cortical ACh release. Theophylline itself increased cortical ACh release, in sympathy with an earlier observation made on brain slices (Vizi & Knoll, 1976).

Theophylline enhancement of cerebral cortical ACh release is probably a result of actions at several levels of the neural axis. The suggestion has been made elsewhere (Phillis and Kostopoulos, 1975; Phillis, Edstrom, Kostopoulos & Kirkpatrick, 1979b) that the firing of central neurones is subject to regulation by endogenously released adenosine. Theophylline would increase the rate of ACh release by antagonizing the depressant action of endogenous adenosine. Theophylline is also a central nervous system stimulant; an action probably related to the antagonism of adenosine at many levels of the neural axis, and as such it arouses the brain. Since cortical ACh release is demonstrably affected by the level of arousal (Mullin & Phillis, 1975; Pepeu, 1973) this property of theophylline must also be considered a significant factor in explaining the theophylline-elicited increase in ACh release.

Methylxanthines are also known to be able to mobilize calcium and to favour transmitter release (Johnson & Inesi, 1969; Berkowitz, Tarver & Spector, 1970; Standaert, Dretchen, Skirboll & Morgenroth, 1976). This could provide an alternative but related explanation for the enhancement of cerebral cortical ACh release by theophylline. The calcium mobilizing effects of theophylline at nerve terminals may yet prove to be a further manifestation of its adenosine antagonist actions, and imply that adenosine stabilizes Ca^{2+} at nerve terminals.

The possibility that diazepam depresses ACh release non-specifically by augmenting the level of anaesthesia appears to be unlikely for various reasons. Diazepam failed to depress release in theophylline pretreated animals, suggesting that its effects are mediated by an adenosine receptor rather than by non-specific alterations in the depth of anaesthesia. Furthermore when given to unanaesthetized mice, diazepam (5 to 40 mg/kg) increased whole brain ACh levels (Consolo, Ladinsky, Peri & Garattini, 1972), the result presumably of a decreased release of ACh.

An interesting finding is the increase in ACh release observed when diazepam was given after theophylline pretreatment. The significance of this observation is uncertain, but it does suggest that diazepam may have more than one action, being capable of both suppressing and enhancing the release of acetylcholine. In this respect there appear to be certain similarities between the actions of diazepam and morphine on ACh release (Phillis, Mullin & Pinsky, 1973).

In conclusion, the results in this paper show that diazepam increases the release of adenosine and its metabolites and depresses the release of ACh from rat cerebral cortex. Its effects on ACh release are abolished by the adenosine antagonist, theophylline. The most plausible explanation for these observations is that diazepam enhances adenosine release by blocking its re-uptake into neuronal and glial cells and that the depression of ACh release is a consequence of these enhanced levels of extracellular adenosine. Adenosine also depresses monoamine release from central and peripheral nerve terminals (Ribeiro, 1978) and a depressant effect of the benzodiazepines on transmitter release at such junctions is to be anticipated. These effects of the benzodiazepines, perhaps in conjunction with a potentiation of the actions of γ-aminobutyric acid, could account for their anxiolytic, anticonvulsant and muscle relaxant actions.

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