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THE SPONTANEOUS AND EVOKED RELEASE OF SPERMINE FROM RAT BRAIN *in vitro*

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1 The efflux of previously accumulated [^{3}H]-spermine from brain slices was measured using a continuous perfusion system. The spontaneous efflux was biphasic, consisting of an initial rapid efflux followed by a much slower release.

2 The slices were depolarized by the addition to the medium of high potassium concentrations, ouabain or veratrine.

3 At concentrations greater than 30 mM, potassium evoked a striking increase in the release of [^{3}H]-spermine. Following uptake in the presence of 5.7×10^{-9} M [^{3}H]-spermine, K^+-evoked release was dependent on the presence of calcium ions. Release of spermine after uptake at 5.6×10^{-8} M or 5.0×10^{-7} M was not calcium-dependent.

4 The calcium-dependent, K^+-stimulated release of spermine was inhibited in the presence of diphenylhydantoin (5×10^{-5} M) or ruthenium red (10^{-5} M).

5 Following uptake of 5.7×10^{-9} M [^{3}H]-spermine in a sodium-free medium, the calcium-dependent, K^+-stimulated release was significantly inhibited.

6 Ouabain (10^{-4} M) caused a large but calcium-independent increase in the efflux of [^{3}H]-spermine.

7 Veratrine-induced release was less substantial but was increased in a calcium-free medium. Release evoked by veratrine was abolished in the absence of sodium.

8 These results are discussed with respect to a possible 'neurotransmitter' or 'neuromodulator' role for spermine.

Introduction

Substantial amounts of the polyamine spermine are present in nervous tissue and the regional variation in brain tissue is much greater than that found in other organs (Kremzner, Barret & Terrano, 1970; Shaw & Pateman, 1973; Seiler & Schmidt-Glenewinkel, 1975). Investigations concerned with elaborating the functions of the polyamines in the central nervous system have recently been reviewed (Shaw, 1979a) and evidence is increasing that these compounds may serve an important role in the brain.

An important criterion for the establishment of a specific neurotransmitter role is that, during stimulation, the substance must be detectable in extracellular fluid collected from the region of the activated synapses (Phillis, 1970; Krnjević, 1974). In more recent times it has been accepted that the study of the release of radiolabelled neurohumoral substances from tissue slice preparations provides useful supporting evidence in satisfaction of this criterion.

To date, the only published study of release of polyamines has employed an indirect method of estimation of the released material in that Russell, Gfeller, Marton & Legendre (1974) have shown that both spermidine and spermine content of Rhesus monkey motor cortex can be decreased by electrical stimulation. The present investigation, which uses a tissue slice preparation, was undertaken in order to obtain more direct evidence that spermine release might be evoked from brain tissue by depolarizing stimuli.

It has recently been reported (Harman & Shaw, 1980a, b) that spermine is actively transported into rat cerebral cortex slices by two high affinity carrier systems. To date, no suitable antagonist of this uptake is available. In order to overcome the problem of re-uptake taking place during the present experiments, a perfusion system similar to that devised by Davies, Johnston & Stephanson (1975) has been used.

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Methods

Incubation of slices

The method of uptake was essentially the same as that previously described (Harman & Shaw, 1980b). In brief, female Wistar rats (125 to 150g) were killed by decaptation and the brains rapidly removed. The cerebral cortex was dissected and chopped at 0.1 mm intervals with the aid of a mechanical chopper (McIlwain & Buddle, 1953) in two directions at 45° and suspended in medium (10 mg/ml). Pre-incubation carried out at 37°C in a shaking water-bath was started by the addition of 0.3 ml tissue suspension to 1.6 ml medium. After 15 min, uptake was started by the addition of spermine (comprising [^{3}H]-spermine (450000 d min^{-1} ml^{-1}) and the required concentration of unlabelled spermine) in a volume of 0.1 ml, and incubation continued for 20 min. The slices were then collected by filtration onto 0.8 μm, 25 mm Millipore membranes contained in 25 ml filtration units (Gelman Hawksley, Northampton, U.K.).

In experiments with [^{3}H]-γ-aminobutyric acid, incubation of slices was carried out for 30 min at 37°C in the presence of 10^{-5} M amino-oxyacetic acid, which was required to inhibit the metabolism of [^{3}H]-γ-aminobutyric acid by GABA transaminase, E.C.2.6.1.19 (Srinivasan, Neal & Mitchell, 1969).

Perfusion of slices

The tissue-impregnated membranes were transferred to filter holders (Swinnex-25, Millipore, U.K.) and immersed in a water-bath at 37°C. Perfusion at a rate of 0.5 ml/min, with medium at 37°C, was achieved with a Gilson Minipuls-2 peristaltic pump. Up to 5 samples of perfusate were collected simultaneously at 2.0 min intervals with a Gilson TDC 220A fraction collector. At the end of the perfusion period, the membranes were allowed to run dry and were then transferred to scintillation vials containing 1.0 ml water. The tissue was solubilized at 37°C for 30 min by the addition of 0.5 ml Protosol (New England Nuclear). The radioactivity of 1.0 ml fractions of perfusate and of the solubilized tissue was measured following the addition of 10 ml scintillant containing 0.5% 2,5-diphenyloxazole and 0.01% 1,4-di [2-(4-ethyl-5-phenyloxazole)]-benzene in a mixture of 2 vol toluene to 1 vol Triton X-100 by liquid scintillation spectrometry using the External Standard Channels Ratio method of quench correction. Acetic acid (50 μl) was added to the tissue samples to reduce chemiluminescence.

Calculation of results

Results are expressed in terms of the fractional rate coefficient (Hopkin & Neal, 1971), which represents the radioactivity released in each fraction expressed as a proportion of that present in the tissue at the time of release.

Materials

Spermine tetrahydrochloride, veratrine and ruthenium red were obtained from the Sigma Chemical Company (Poole, Dorset). Ouabain was from BDH (Atherstone, Warwicks.).

[^{3}H]-spermine tetrahydrochloride (44.28 Ci/mmol) was purchased from New England Nuclear, GmbH; [^{14}C]-urea (60 mCi/mmol) and [^{3}H]-γ-aminobutyric acid (54 Ci/mmol) were obtained from the Radiochemical Centre, Amersham).

The usual incubation medium had the following composition (mM): NaCl 119, glucose 10, $NaHCO_3$ 25, KCl 5, $MgSO_4$. $7H_20$ 1.2, NaH_2. $2H_20$ 1 and $CaCl_2$ 0.75 and was gassed with a mixture of 5% CO_2 in O_2 for at least 20 min before use (McIlwain, Harvey & Rodriguez, 1969).

In perfusions with sodium-free medium, the sodium hydrogen carbonate content was replaced by 25 mM Tris buffer, and sodium chloride was replaced by an isoosmotic equivalent of sucrose.

Results

Spontaneous efflux

The release of [^{3}H]-spermine from rat cerebral slices was biphasic (Figure 1), and, following uptake at 5.7×10^{-9} M, consisted of an initial rapid efflux and a slower component. The initial phase was complete within 20 min of the start of the perfusion at this concentration of spermine. At initial medium concentrations of 5.6×10^{-8} M and 5×10^{-7} M [^{3}H]-spermine. this washout phase was also complete within 20 min of the start of the perfusion. The initial rate of washout of spermine was the same in both normal and calcium-free medium (Table 1). In subsequent experiments, where depolarizing stimuli were applied, these were applied at 26 and 40 min to avoid the initial phase of efflux. In experiments using [^{3}H]-GABA, the initial rate of efflux was faster, and was complete within 10 min of the start of the perfusion.

At 5.7×10^{-9} M [^{3}H]-spermine, the basal rate of efflux was greater in normal than calcium-free medium (Table 1) and of a similar order to that calculated for [^{3}H]-GABA. Moreover, the basal rate of efflux at both the higher concentrations of spermine employed was similar to that described for [^{3}H]-spermine efflux using an initial medium concentration of 5.7×10^{-9} M in calcium-free medium.

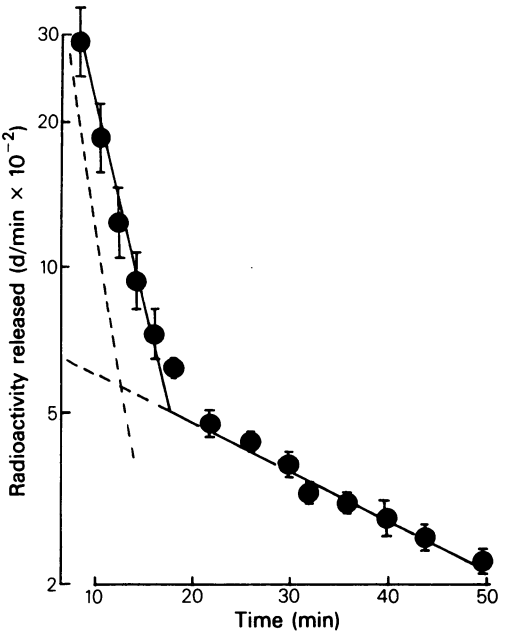

Figure 1 Spontaneous efflux of [^{3}H]-spermine from cortical slices. Each point is the mean of five experiments. The second phase of efflux was extrapolated and the release obtained from this line was subtracted from the combined release curve to give values for the initial rapid phase of efflux (broken line).

Effect of high potassium concentrations on efflux

In experiments with high potassium concentrations, the depolarizing agent was added to the perfusing medium for 4 min.

Experiments were carried out in medium in which an isoosmotic equivalent of sodium was removed to compensate for the high potassium concentration. At an initial uptake concentration of 5.7×10^{-9} M [^{3}H]-spermine, depolarization in a decreased-sodium medium evoked the release of 9.4 ± 1.2% (mean ± s.e. mean, n = 5) of the total tissue radioactivity, more than doubling the rate of efflux. The period of release outlasted the period of stimulation by approximately 6 min (Figure 2). In calcium-free medium, the amount of spermine released was decreased by approximately 71%, and comprised 2.7 ± 1.1% (n = 5) of the total tissue radioactivity. The addition of 2 mM ethylene-diaminetetraacetic acid (EDTA) throughout the perfusion had no significant effect on calcium-independent efflux (2.6 ± 0.9%, n = 5, total tissue radioactivity released).

The second application of 50 mM K^+ produced an increase in the rate of efflux of spermine which was only 27% as great as the response to the first application but which was again partially dependent on the presence of calcium ions (Figure 2). The extent of calcium-dependence of this second response was smaller than that of the first.

The total tissue radioactivity released was dependent on the length of the stimulation period, and the strength of the stimulus. With 50 mM K^+, the amount of [^{3}H]-spermine released increased from 2.8 ± 0.5% (n = 4) following a stimulation period of

Table 1 Spontaneous release rate constants of spermine and γ-aminobutyric acid (GABA)

	1st order rate constants (min^{-1}) k_1	k_2
5.7×10^{-9} M [^{3}H]-spermine:		
Normal	0.274 ± 0.012	0.023 ± 0.001
Ca^{2+}-free	0.270 ± 0.007	0.016 ± 0.003†
5.6×10^{-8} M [^{3}H]-spermine:		
Normal	0.273 ± 0.003	0.016 ± 0.002
Ca^{2+}-free	0.354 ± 0.025	0.017 ± 0.002
5.0×10^{-7} M [^{3}H]-spermine:		
Normal	0.385 ± 0.002	0.013 ± 0.0004
Ca^{2+}-free	0.333 ± 0.008	0.015 ± 0.001
5.0×10^{-7} M [^{3}H]-GABA:		
Normal	0.394 ± 0.010	0.020 ± 0.001
* 1.0×10^{-7} M [^{3}H]-GABA		
Normal	0.800	0.011

† $P<0.05$
* Data from Srinivasan *et al.* (1969)
Each result is the mean ± s.e. mean of 5 experiments.

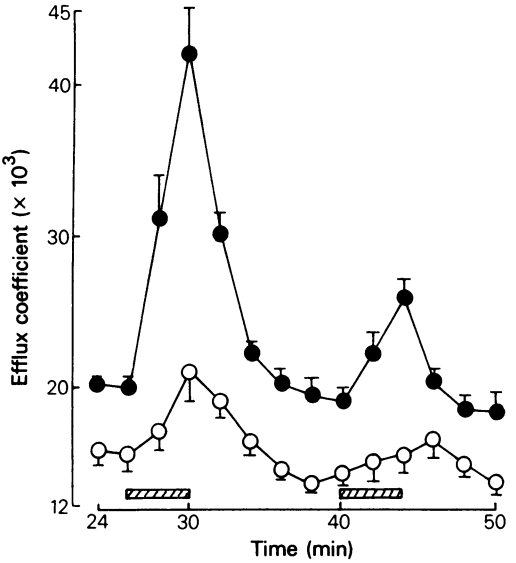

Figure 2 The effect of high potassium concentration (50 mM, isotonic) on the efflux of [^{3}H]-spermine, from brain slices in normal (●) and in calcium-free (○) medium. Each point is the mean of five experiments; vertical lines show s.e. mean. The periods of high potassium perfusion are indicated by the hatched areas.

2 min to 14.1 ± 0.8% (n = 5) of the total tissue radioactivity when the stimulation was extended to 6 min duration. In addition, significant amounts of release of [^{3}H]-spermine were only achieved at a potassium concentration equal to, or greater than, 30 mM. At a concentration of 20 mM K^+ or below, less than 2% of the total tissue radioactivity was released whilst 30 mM K^+ released 3.2 ± 1.0% (n = 10) of the total tissue radioactivity.

Experiments were also carried out in a medium containing 50 mM K^+ which had not been rendered isotonic. Depolarization in a hypertonic solution produced a peak which was less sharp than that produced in an isotonic solution, but released a total radioactivity which was not significantly different (7.9 ± 0.9%, n = 7) from that previously released by an isotonic medium.

Release of radioactivity caused by high potassium concentrations was also studied following uptake of higher concentrations of spermine. Following incubation in the presence of 5.6 × 10^{-8} M [^{3}H]-spermine, K^+-evoked release was independent of the presence of calcium ions, releasing 4.9 ± 0.6% (n = 5) of the total tissue radioactivity. After uptake at 5 × 10^{-7} M [^{3}H]-spermine, 3.9 ± 1.2% (n = 4) of the total tissue radioactivity was released by 50 mM K^+, a response which was also not significantly calcium-dependent.

Effect of diphenylhydantoin on release evoked by 50 mM K^+

Diphenylhydantoin was added to the medium 8 min after the start of the perfusion and maintained in the medium through the period of stimulation (Minchin & Iversen, 1974). Following uptake at 5.7 × 10^{-9} M, diphenylhydantoin (5 × 10^{-5} M) produced an 88% decrease in the K^+ stimulated release of spermine (Figure 3) whilst 5 × 10^{-6} M diphenylhydantoin produced a 32% decrease which was a statistically insignificant effect (P>0.05). At the higher concentration the basal rate of efflux was decreased (Figure 3).

Effect of ruthenium red on release evoked by 50 mM K^+

Preliminary experiments indicated that ruthenium

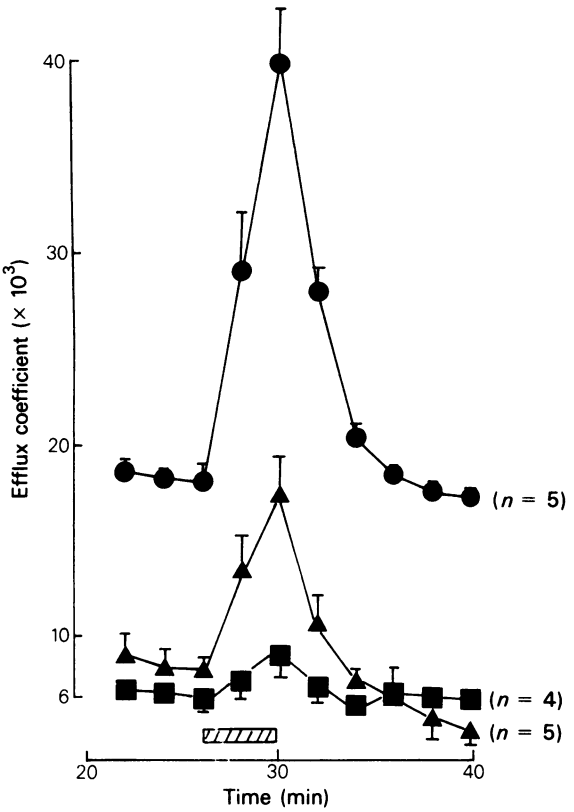

Figure 3 The effects of diphenylhydantoin (■) and ruthenium red (▲) on K^+-stimulated (50 mM) efflux of [^{3}H]-spermine (●) from brain slices. Diphenylhydantoin (5 × 10^{-5} M) was perfused in the medium from 8 min to 30 min after the start of the perfusion. Ruthenium red (1 × 10^{-5} M) was present only during the uptake period. Each result is the mean of the number of experiments indicated in parentheses; vertical lines show s.e. mean.

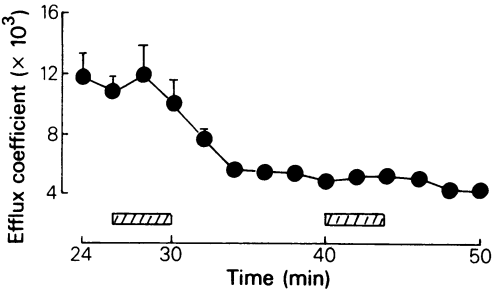

Figure 4 The effect of high potassium (50 mM), isotonic) on the efflux of [^{14}C]-urea, from brain slices in normal medium. The periods of high potassium perfusion are indicated by the hatched areas. Each result is the mean of five experiments; vertical lines show s.e. mean.

red, when added in a concentration up to 1×10^{-5} M, did not inhibit the uptake of 1.1×10^{-9} M [^{3}H]-spermine into rat cerebral cortex slices. Following uptake in the presence of 1×10^{-5} M ruthenium red, the release of [^{3}H]-spermine (5.7×10^{-9} M) evoked by 50 mM K^+ was depressed by 62.8 ± 16.8% (n = 5) (Figure 3). The basal rate of efflux was again decreased.

Uptake in sodium-free medium: effect on release evoked by 50 mM K^+

Following uptake of 5.7×10^{-9} M [^{3}H]-spermine in a medium in which its sodium content had been replaced by an isoosmotic equivalent of sucrose, a depolarizing concentration of potassium was applied as described previously. The response to the first stimulation was reduced by 66% following uptake in sodium-free medium, whereas the response to the second stimulation was not significantly affected.

Perfusions with [^{14}C]-urea

Slices of rat cerebral cortex were perfused as described previously following 20 min incubation with 6.5×10^{-5} M [^{14}C]-urea. High potassium concentrations failed to produce a significant efflux of this substance from brain slices (Figure 4).

Effect of ouabain on efflux

The increased rate of efflux of [^{3}H]-spermine evoked by 1×10^{-4} M ouabain was independent of the presence of calcium ions (Figure 5). A 2.5 to 3 fold increase in the efflux rate, releasing a total of 8.2 ± 1.0% (n = 8) in normal and 8.6 ± 0.2% (n = 8) in calcium-free medium, was observed. In both experiments, the period of release only outlasted the period of stimulation by 6 min. The second application of ouabain produced a response which was greatly decreased in both normal and calcium-free medium.

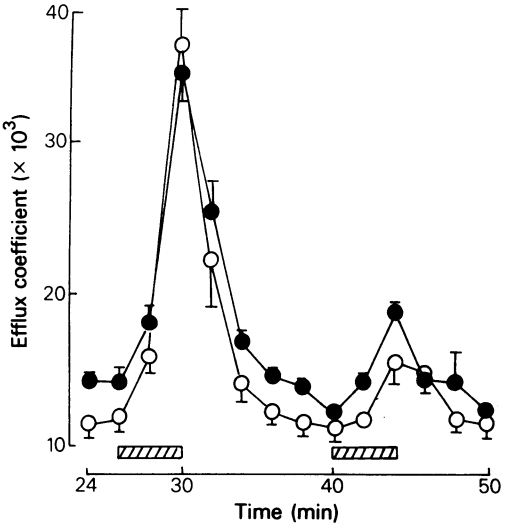

Figure 5 The effect of ouabain 10^{-4} M on the efflux of [^{3}H]-spermine, from brain slices, in normal (●) and calcium-free medium (○). The periods of perfusion with ouabain are indicated by the hatched areas. Each result is the mean of eight experiments; vertical lines show s.e. mean.

Effect of veratrine on efflux

Release evoked by 10 μg/ml veratrine was less substantial than that produced by other depolarizing agents, comprising only 5.3 ± 1.7% (n = 9) of the total tissue radioactivity. A sustained increase in the rate of efflux occurred in calcium-free medium (Figure 6).

Following perfusion of rat cerebral cortex slices in a sodium free medium, the release of 5.7×10^{-9} M [^{3}H]-spermine evoked by 10 μg/ml veratrine was completely abolished (Figure 6).

Discussion

The spontaneous release of [^{3}H]-spermine from 0.1 mm slices of rat cerebral cortex is biphasic, consisting of an initial rapid phase, which presumably represents washout of loosely bound extracellular material, and a slower phase which represents the basal rate of efflux. A similar pattern of biphasic release occurs with [^{3}H]-noradrenaline (Baldessarini & Kopin, 1966; 1967) and [^{3}H]-GABA (Srinivasan *et al.*, 1969) from brain slices, and with [^{14}C]-glutamate from dorsal root ganglia (Minchin & Iversen, 1974). This pattern of biphasic release, associated with marked retention of radioactivity has been suggested as being consistent with the view that the material taken up by the tissue mixes with the endogenous pool (Srinivasan *et al.*, 1969; Hopkin & Neal, 1971). In the

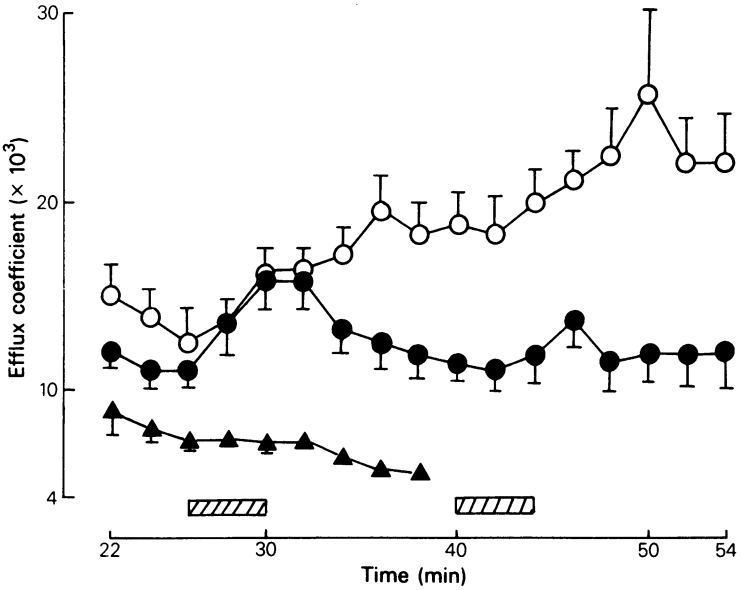

Figure 6 The effects of veratrine 10 μg/ml on the efflux of [^{3}H]-spermine from rat cerebral cortex slices. Perfusions were carried out in normal (●), calcium-free (○) or sodium-free medium (▲). The period of stimulation with veratrine is indicated by the hatched areas, and mean results are shown (n = 9); vertical lines show s.e. mean.

present experiments when Millipore membranes were used to collect the slices, the initial rate of spermine washout was the same in both normal and calcium-free medium, and was complete within 20 min of the start of the perfusion. However, the GABA washout rate was somewhat faster, and was complete after 10 min. In similar experiments carried out by Srinivasan *et al.* (1969) using Whatman glass fibre filters, the washout rate for GABA was approximately double that found in these experiments, although the basal rate of efflux is of the same order. The slower washout for spermine is probably related to the inherent tendency of this polyamine to bind to Millipore membranes, (Tabor & Tabor, 1964) although this association is negligible in comparison to the interaction of polyamines with glass fibre filters.

It is important to establish that the radioactivity present in the perfusate is likely to consist largely of unchanged spermine. Interconversion of the polyamines is known to occur through the action of diamine oxidase (EC 1.4.3.6.) whose action has been demonstrated in mouse (Fischer, Korr, Seiler & Werner, 1972) and rabbit brain (Halliday & Shaw, 1976), though the half-life of spermine in brain is long (Shaw, 1979b; Antrup & Seiler, 1980).

In rat cortex slices following uptake at 5.7×10^{-9} M [^{3}H]-spermine and separation of polyamines by high pressure liquid chromatography (Shaw, Al-Deen & Elworthy, 1980) some 7% of the radioactivity originally associated with the spermine peak was transferred to the putrescine fraction but 85% of the total radioactivity remained in the spermine fraction.

In the present experiments the presence of less than 150 counts per min per sample of perfusate precludes accurate determination of the proportion of spermine actually present in the perfusate but it is assumed that the radioactivity is largely associated with spermine.

The rate of release of [^{3}H]-spermine is increased by the addition to the perfusion medium of high concentrations of potassium ions. Elevated external potassium concentrations have also been shown to increase the release from brain slices of noradrenaline (Baldessarini & Kopin, 1967); GABA (Machiyama, Balazs & Richter, 1967; Srinivasan *et al.*, 1969; Leach, 1979), D-aspartate (Davies & Johnston, 1976), glycine (Hopkin & Neal, 1971) and taurine (Kaczmarek & Davison, 1972). Evidence that the release of [^{3}H]-spermine from brain slices is not due to an indiscriminate increase in membrane permeability is indicated by the inability of high potassium concentrations to evoke the release of [^{14}C]-urea. In addition, since the amount of spermine released in a medium in which the sodium concentration was decreased by an isoosmotic equivalent was not significantly different from that induced in a medium whose composition had not been adjusted, potassium-evoked release cannot be attributed to hypertonicity.

Most known neurohumoral mechanisms of release are calcium-dependent (Kelly, Deutsch, Carlson & Wagner, 1979). Following potassium depolarization, there is a very rapid, almost instantaneous increased uptake of calcium by nerve terminals, followed by an uptake rate which approximates that seen in non-depolarized terminals (Blaustein & Weissmann, 1970). The potassium-induced release of spermine is only found to be calcium-dependent at an initial spermine uptake concentration of 5.7×10^{-9} M [^{3}H]-spermine. At this concentration, approximately 85% of the uptake occurs via a 'very high' affinity system ($Km = 3.7 \times 10^{-9}$ M) (Harman & Shaw, 1980b). On increasing the spermine uptake concentration, release is no longer calcium-dependent. In addition, the percentage of the total tissue radioactivity released decreases with increasing spermine concentrations.

It is evident that the calcium-dependent pool of spermine release which may represent a neuronal system is rapidly depleted. The response to a second stimulation with 50 mM potassium in slices which have accumulated spermine at a concentration of 5.7×10^{-9} M is 73% less than that evoked by the first stimulation. The fact that some release of spermine still occurred in a calcium-free medium may be attributed to a supply of calcium from intraterminal storage sites (Lowe, Richardson, Taylor & Donatsch, 1976). However, the finding that ethylenediaminetetraacetic acid (EDTA) did not further decrease the release of spermine tends to suggest that another mechanism is responsible for this small calcium-independent component.

Further evidence that calcium does play an important role in the release of spermine evoked by potassium derives from the observation that diphenylhydantoin reduced potassium-evoked release. The influx of calcium into synaptosomes is inhibited by diphenylhydantoin (Sohn & Ferrendelli, 1973), as is the release of noradrenaline from potassium-depolarized brain slices (Pincus & Lee, 1972).

Ruthenium red, an inorganic dye, is a specific inhibitor of calcium transport into mitochondria (Moore, 1971; Rahimimoff & Alnaes, 1973; Reed & Bygrave, 1974) and has also been shown to prevent the calcium-dependent release of GABA from mouse brain synaptosomes (Tapia & Maeza-Ruix, 1977). Similarly, at a concentration that does not prevent the uptake of [^{3}H]-spermine into rat cerebral cortex slices, ruthenium red inhibited the calcium-dependent release of spermine (5.7×10^{-9} M [^{3}H]-spermine) evoked by potassium. It is interesting to note that the percentage inhibition of release produced by ruthenium red ($62.8 \pm 16.8\%$, $n = 5$) is a value very close to that produced by perfusion with a calcium-free medium, indicating the specificity of the action of ruthenium red on calcium transport.

The increased calcium influx into nerve terminals associated with the process of depolarization is only seen when the potassium concentration is increased above 15 to 20 mM (Blaustein, 1975), increasing to a maximum influx of calcium at a concentration of potassium of 60 mM. In the present experiments, significant increase in the efflux of [^{3}H]-spermine only occurred at potassium concentrations of 30 mM or more. Nevertheless, in calcium-free medium or in the presence of diphenylhydantoin or ruthenium red, spontaneous release was greatly reduced. A calcium-dependent component therefore also plays a part in spontaneous release.

Evidence for the suggestion that the sodium-dependent uptake of [^{3}H]-spermine at low concentrations may be associated with a possible transmitter pool was examined by measuring the K^+-stimulated release of spermine from rat cerebral cortex slices which had been labelled with [^{3}H]-spermine in the absence of sodium. The release of spermine following uptake in a sodium-free medium was decreased by 66%, a value which again closely corresponds to the degree of inhibition observed when perfusions were performed in a calcium-free medium. This evidence, together with that derived from the experiments with ruthenium red, lends strong support for the suggestion that the calcium-dependent release of spermine from rat cerebral cortex slices may in fact represent release from a neuronal, and possibly a 'transmitter', pool.

Depolarization of nervous tissue can also be induced by agents which increase the intracellular sodium content of nerve terminals either directly or indirectly.

Ouabain causes release through inhibition of the Na^+, K^+-activated adenosine triphosphatase (Na^+, K^+-ATPase) system (Skou, 1960; Kimmich, 1970; Archibald & White, 1974; Duncan, 1977). The release of [^{3}H]-spermine evoked by 10^{-4} M ouabain was approximately equivalent to the total released by 50 mM K^+, and ouabain-evoked release was independent of the presence of calcium ions. Ouabain-evoked release of amino acids (Benjamin & Quastel, 1972) and GABA (Raiteri, Federico, Coletti, & Levi, 1975; Leach, 1979) has also been demonstrated.

The role of veratrine, and the veratrum alkaloids, in the release of suspected transmitter agents, has recently been reviewed by Minchin (1980). The release of spermine evoked by veratrine was not found to be calcium-dependent, and, in addition, the releasing potency of veratrine was considerably less than that of the other depolarizing stimuli employed. This may be due in part to the impurities present in veratrine, which consists of a mixture of five alkaloids, two of which are veratridine and cevadine. There is also some confusion as to the role of calcium in the release evoked by veratrum alkaloids. Thus, the release of acetylcholine from rat brain slices

(Grewaal & Quastel, 1973) and noradrenaline from synaptosomes (Blaustein, Johnson, & Needleman, 1972) has been found to be decreased in the absence of calcium ions, whilst that of glutamate and aspartate (Benjamin & Quastel, 1972) and GABA (Benjamin & Quastel, 1972; Neal, 1979; Neal & Bowery, 1979; Szerb, 1979) was found to be enhanced. In experiments with veratrine, a persistent increase in the rate of efflux of [^{3}H]-spermine occurred in calcium-free medium. The veratrum alkaloids are thought to cause depolarization by increasing the permeability of the membrane to sodium ions (Shanes, 1963; Baker, 1968), thus increasing the intraterminal sodium concentration. Thus, veratridine increases the sodium content of synaptosomes (Li & White, 1977) and protoveratrine A increases sodium uptake into brain slices (Okamoto & Quastel, 1970). In the absence of calcium ions, this influx of sodium ions is facilitated and prolonged (Frankenhauser & Hodgkin, 1957; Hille, 1968). This observation may therefore account for the persistent increase in the efflux of spermine observed in a calcium-free medium.

Further indication that the depolarization induced by veratrine is a result of influx of sodium is the finding that this response is abolished in a sodium-free medium. A similar response has been observed with the 'neuronal' release of [^{3}H]-GABA (Neal & Bowery, 1979).

At the highest initial spermine concentration employed (5×10^{-7} M [^{3}H]-spermine) approximately 75% of uptake occurs via a 'high' affinity system ($Km = 5.8 \times 10^{-7}$M). Since the uptake of 5.7×10^{-9}M [^{3}H]-spermine is sodium-dependent, temperature-dependent and strongly susceptible to inhibition by metabolic inhibitors (Harman & Shaw, 1980b), properties which are consistent with neuronal transport, the release of spermine at this concentration in response to raised potassium concentrations may possibly reflect the depolarization of neuronal elements. Evidence is increasing that the potassium-evoked release of GABA from preparations which are rich in glia is independent of the presence of calcium ions (Sellstrom & Hamberger, 1977; Neal & Bowery, 1979) though Minchin & Iversen (1974) have reported calcium-dependent release from glia in rat dorsal root ganglia. It is possible that the release of spermine, perhaps especially after uptake at the higher concentrations, may represent a glial process since polyamines are associated with both glial and neuronal elements (Fischer *et al.*, 1972). However, it has been suggested that in slices chopped at 0.1 mm intervals glia may form only a very minor component (Riddall, Leach & Davison, 1976). The question as to the localization of the releasable spermine pools must, at present remain open but investigation of the uptake and release of spermine in 0.4 mm, rather than 0.1 mm slices may provide further insight into this problem.

To conclude, there is now increasing evidence which strongly supports a neurohumoral role for spermine. The present findings indicate that spermine release from rat cortex slices follows the classical pattern associated with the release of established neurotransmitters. There is also evidence that polyamines are active in influencing the firing rate of rat and cat brain stem neurones (Wedgwood & Wolstencroft, 1977) and the high affinity uptake system for spermine recently described (Harman & Shaw, 1980b) provides a means by which released spermine could be inactivated.

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