

Nicotinamide adenine dinucleotide depresses synaptic transmission in the hippocampus and has specific binding sites on the synaptic membranes

C.D. Richards, C.R. Snell* & P.H. Snell*

Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, and the MRC Neuroendocrinology Unit*, Newcastle General Hospital, Newcastle upon Tyne NE4 6BE

1 The electrical activity of transverse slices of hippocampus was used as a bioassay in which extracts of fresh brain tissue were screened for biological activity. A factor that depressed synaptic transmission was identified as nicotinamide adenine dinucleotide (NAD). This depressant action of NAD could be observed at concentrations in the range 1–10 μ M and the degree of depression was monotonically related to the concentration of NAD in the bathing medium.

2 NAD did not affect the antidromic invasion of the granule cells nor did it alter the relationship between the electrically evoked excitatory postsynaptic field potential (e.p.s.p.) and the population discharge of the granule cells (population spike). These results suggest that NAD did not affect the electrical excitability of the neuronal membranes.

3 NAD had little effect on the sensitivity of granule cells to iontophoretically applied L-glutamate, the putative excitatory transmitter for the perforant path-granule cell pathway.

4 Pure synaptosomal membranes, free of mitochondria, had two binding sites for NAD: a high affinity site with a K_d of 1 μ M and a low affinity site with a K_d of 17 μ M. These sites were similar in affinity to those of mitochondria, although the density of the high affinity sites was 5 \times greater in the synaptosomal membranes. Adenosine had a relatively weak affinity for the NAD binding sites.

5 It was concluded that NAD probably depressed synaptic transmission in the dentate gyrus by binding to sites on the presynaptic nerve terminal and reducing the amount of transmitter released by a nerve impulse. The physiological significance of this view is discussed.

Introduction

Recent studies have shown that many biologically active substances are present in the brain. Although their precise physiological roles remain largely unknown, several lines of evidence suggest that many are neuromodulators or synaptic transmitters in specific regions of the brain (see Krnjević, 1974).

Since the levels of many of these active materials are low and since their distribution is often highly localized to specific structures and pathways (e.g. substance P, Cuello & Kanazawa, 1978) there is a strong possibility that many other neuroactive materials remain to be discovered. However, the search for novel neuroactive materials requires the development of a suitable bioassay, preferably one that is based on a physiological process that occurs within the CNS itself. The use of brain tissue slices for electrophysiological studies initiated by Yamamoto

& McIlwain (1966) and subsequently developed by others (see Richards, Smaje & White 1976) offers suitable opportunities. In an earlier communication to the Physiological Society we described the use of transverse slices of the hippocampus for the bioassay of materials isolated from fresh brain tissue and gave evidence that an unknown material present in our extracts, possibly a peptide, could reversibly depress synaptic transmission in the dentate gyrus of the hippocampus (Richards & Snell, 1979). Here we show that nicotinamide adenine dinucleotide (NAD) is mainly responsible for this depressant activity and we further show that the NAD binding sites in the synaptic membranes are distinct from those for adenosine. A preliminary communication has been given to the Physiological Society (Richards, Snell & Snell, 1983).

Methods

Assay procedure

The assay used was based on the transverse slice of the hippocampus first described by Skrede & Westgaard (1971). Precise details of the preparation of the slices have been given elsewhere (Richards, 1981). For the assay, transverse slices of guinea-pig hippocampus, 300 μm thick, were cut perpendicular to the long axis of the hippocampus and were subsequently incubated in the chamber described by Richards & Tegg (1977). This chamber permits the slice to be superfused by a stream of oxygenated artificial cerebrospinal fluid (a.c.s.f.) over both surfaces. After allowing 30–60 min for the slices to recover from the trauma of isolation, recordings were made from the granule cell layer of the dentate gyrus. Electrical stimulation of the slices was effected by a pair of tungsten wire electrodes which were insulated except at their tips. They were placed in the perforant path adjacent to the hippocampal fissure. The stimulation pulses of 10–20V, 50–100 μs duration were

derived from an optically isolated stimulator driven by a Digitimer at a rate of one pulse every 3 to 12 s. The evoked synaptic potential and population cell discharge was recorded from the granule cell layer 1–2 mm from the stimulating electrode by means of glass microelectrodes (tip diameter 2–5 μm) filled with 0.5M NaCl. The potentials were recorded monopolarly, the indifferent electrode being a silver-silver chloride wire placed in the fluid of the recording chamber. Conventional methods of amplification and display were used except that the evoked potentials were stored in the memory of a digital oscilloscope (Gould 4000) before being written out on a chart recorder. To assay the various fractions isolated from the brain tissue the flow of a.c.s.f. was stopped for 30–60 s before addition of the sample. Samples were added directly to the medium bathing the slice by means of a 25 μl micropipette. After the addition of the test sample, the flow of the bathing medium remained stopped for a further 1–2 min while the effect of the sample on the evoked potentials was followed. The flow was then re-established to wash away the added material. All samples were dissolved

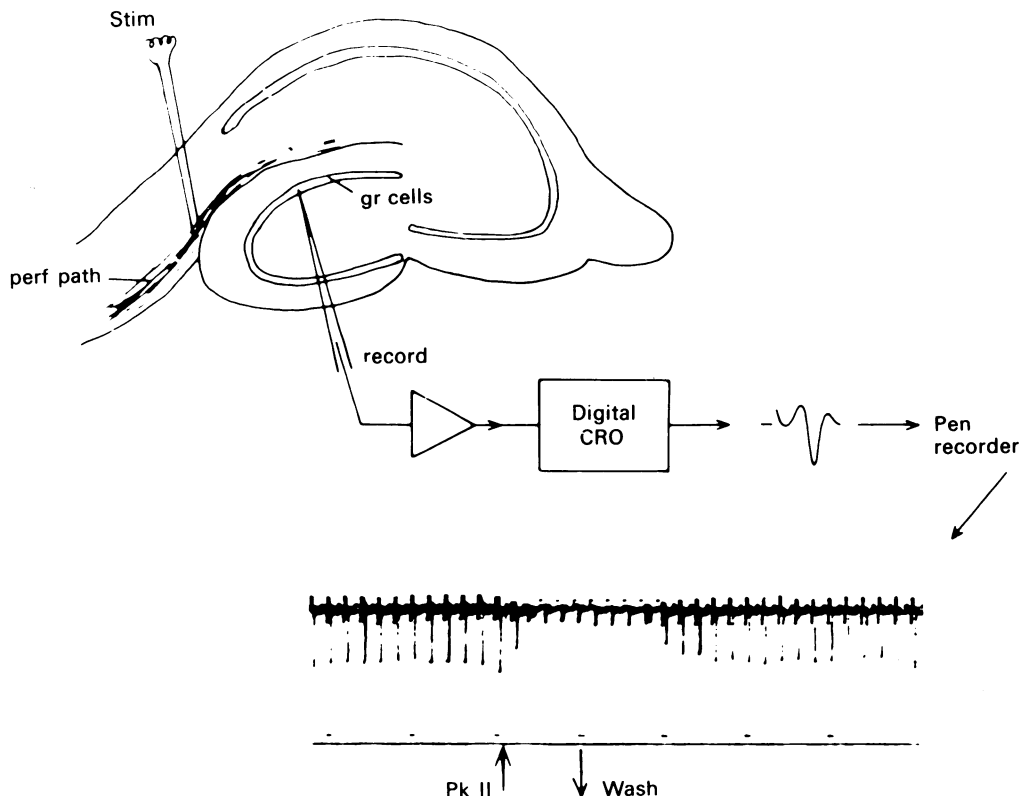


Figure 1 Schematic drawing of the assay system. The original record at the foot of the figure shows sharp downward deflections which represent the peak amplitude of the population spike. The time calibrations are 1 min apart (lower trace). For further details see text. Stim = stimulating electrode; gr cells = granule cells; perf path = perforant path.

in a.c.s.f. and their pH checked before assay. A schematic drawing of the assay is shown in Figure 1.

The a.c.s.f. used to bathe the preparations had the following composition (mM): NaCl 134, KCl 5, KH_2PO_4 1.25, CaCl_2 1, MgSO_4 2, NaHCO_3 16 and glucose 10. It was saturated with 95% O_2 : 5% CO_2 before used and had a pH of 7.35–7.4 at 37°C.

Iontophoresis

Single glass microelectrodes (1–2 μm tip, 4–8 M Ω resistance) were inserted into the granule cell layer of the dentate gyrus to record the discharge of a single neurone evoked by perforant path stimulation. This achieved, a three barrelled electrode was inserted in the region of the molecular layer innervated by the perforant path. Each barrel contained one of the following solutions: sodium glutamate (0.2 M), sodium aspartate (0.2 M) or sodium chloride (0.2 M). Glutamate or aspartate was ejected from the iontophoretic (3-barrelled) electrode in brief pulses of about 300 nA for 1 s while the iontophoretic electrode position was adjusted for an optimal response. Thereafter its position was unchanged throughout a given test. For the test pulses of 100–200 nA, 100–500 ms were used with current controls applied from the barrel filled with sodium chloride. The response of a cell to glutamate or aspartate was monitored before, during and after application of NAD and results displayed as post-stimulus time histograms.

Isolation of neuroactive material

Guinea-pigs were stunned by a blow to the back of the neck and their spinal cord severed before removal of the brain. Immediately after removal the brains were dropped into acid acetone cooled to –80°C. Subsequently the brains were homogenized in the acid acetone at 0°C and the resulting suspension stirred overnight at 4°C. The supernatant was separated by filtration and the residue re-extracted with 50% acetone. The combined extracts were evaporated to dryness and the residue dissolved in 50% acetic acid. The fat was removed from the main body of the liquor by centrifugation and the remaining sample was subjected to gel filtration on a Sephadex G15 column (2.5 \times 100 cm) equilibrated with 50% acetic acid. Eluate fractions were then assayed for biological activity as indicated above. A powerful depressant activity was detected running just behind the material excluded from the gel (see Results). This activity was then purified further by high voltage paper electrophoresis on Whatman 3MM paper at pH 2.8 (1M acetic acid; 3kV, 30 min) and then again at pH 6.3 (pyridine-acetic acid water 2.3:0.6:97 2kV, 45 min). In each case the paper was dried,

divided into seven zones and the individual zones eluted with 10 ml 1M acetic acid. The resulting liquor was evaporated to dryness before assay. Final purification was achieved with high pressure liquid chromatography (h.p.l.c.) on a Waters C_{18} reverse phase column (0.6 \times 25 cm) in 2 mM HCl at a flow rate of 1 ml min⁻¹. The effluent from the column was collected in 1 ml fractions and each was assayed for biological activity as before. The activity was found to be in a single peak which was subsequently identified as nicotinamide adenine dinucleotide (see Results).

Preparation of synaptosomal membranes and mitochondria

Twelve rat brains were homogenized in 10 times their volume of 0.32 M sucrose and centrifuged at 1000 g for 10 min. The supernatant was removed and recentrifuged at 50,000 g for 25 min. The resulting pellet was rehomogenized in 0.01 M Tris-HCl buffer pH 7.4 (60 ml) and left at room temperature for 60 min. The homogenate was then diluted with 2 volumes of 48% w/w sucrose and 28.5% (w/w) sucrose layered on top before centrifugation at 30,000 g for 20 min. Lysed synaptosomal membranes were collected from the interface, diluted with 2 volumes of 0.01 M Tris pH 7.4 and centrifuged at 50,000 g for 30 min. The resulting pellet of synaptosomal membranes was washed three times and stored at –70°C in 2 ml aliquots. Mitochondria were collected as the pellet from the discontinuous sucrose gradient centrifugation, suspended in 0.01 M Tris pH 7.4 and, as for the synaptosomal membranes, washed 3 times with the same buffer before storage at –70°C in 2 ml aliquots.

Total protein content of the synaptosomal membrane and mitochondria was determined by the method of Lowry Rosebrough, Farr & Randall (1951).

[³H]-nicotinamide adenine dinucleotide displacement assay

Mitochondria or synaptosomal membranes (0.5 mg protein) were preincubated with [³H]-NAD (26.1 Ci mmol⁻¹; N.E.N. Inc. 1.8 pmol per tube) for 2 min at room temperature before being added to a serial dilution of the relevant unlabelled ligand in a final volume of 1 ml 0.5 M Tris, 1% BSA pH 7.4. After a further 30 min at 1°C the tubes were centrifuged at 14,000 g for 2 min to separate the bound from the free label. The pellets were superficially washed with ice cold buffer (0.2 ml) and suspended in water (0.5 ml). Packard 299 Scintillant (6 ml) was added and the radioactivity in the pellet measured using a Packard TriCarb 460CD instrument with on-line d min⁻¹ correction.

Specific binding was calculated as the difference

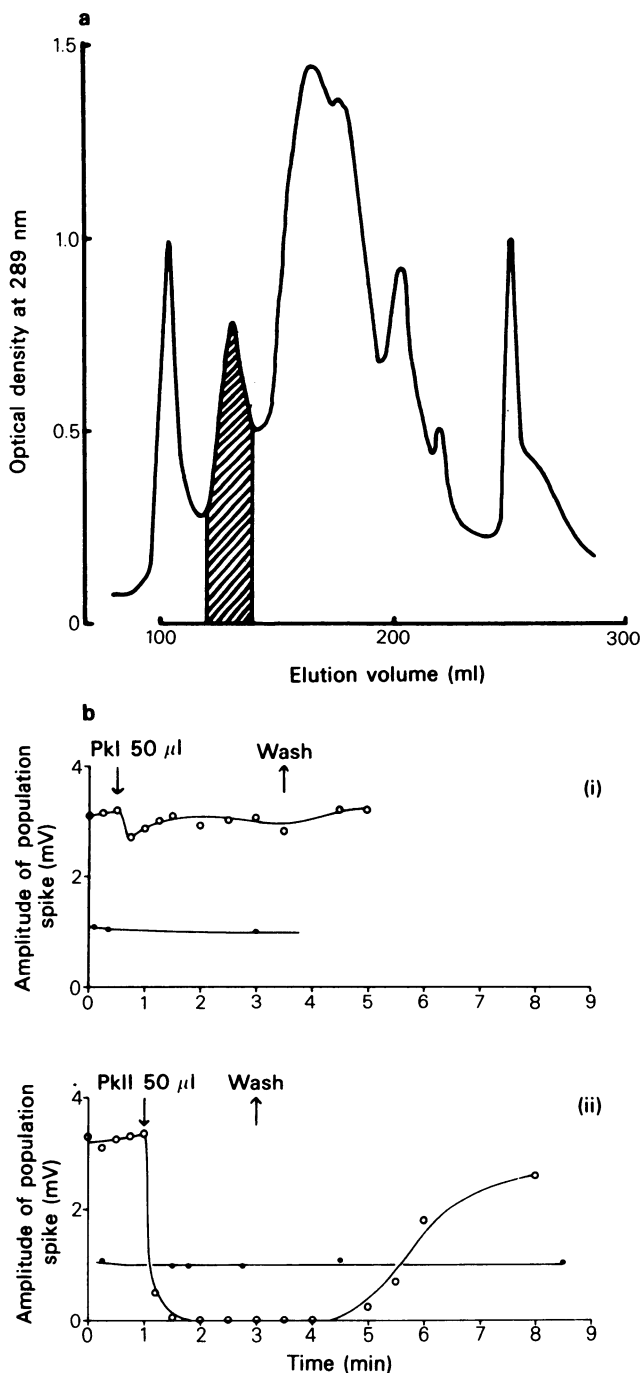


Figure 2 (a) Elution profile of the acid-acetone extract of brain tissue chromatographed on a Sephadex G15 column. Column dimensions 100×2.5 cm, eluent: 50% glacial acetic acid. The shaded area shows the greatest concentration of depressant activity. (b) Examples from the assay of the fractions from the G15 column. (i) The effect of fractions from the first peak on the amplitude of the orthodromic (○) and antidromic (●) population spikes. (ii) The effect of a fraction from the region represented by the shaded area represented in (a). Key as for (i). Note the lack of effect on the antidromic population spike.

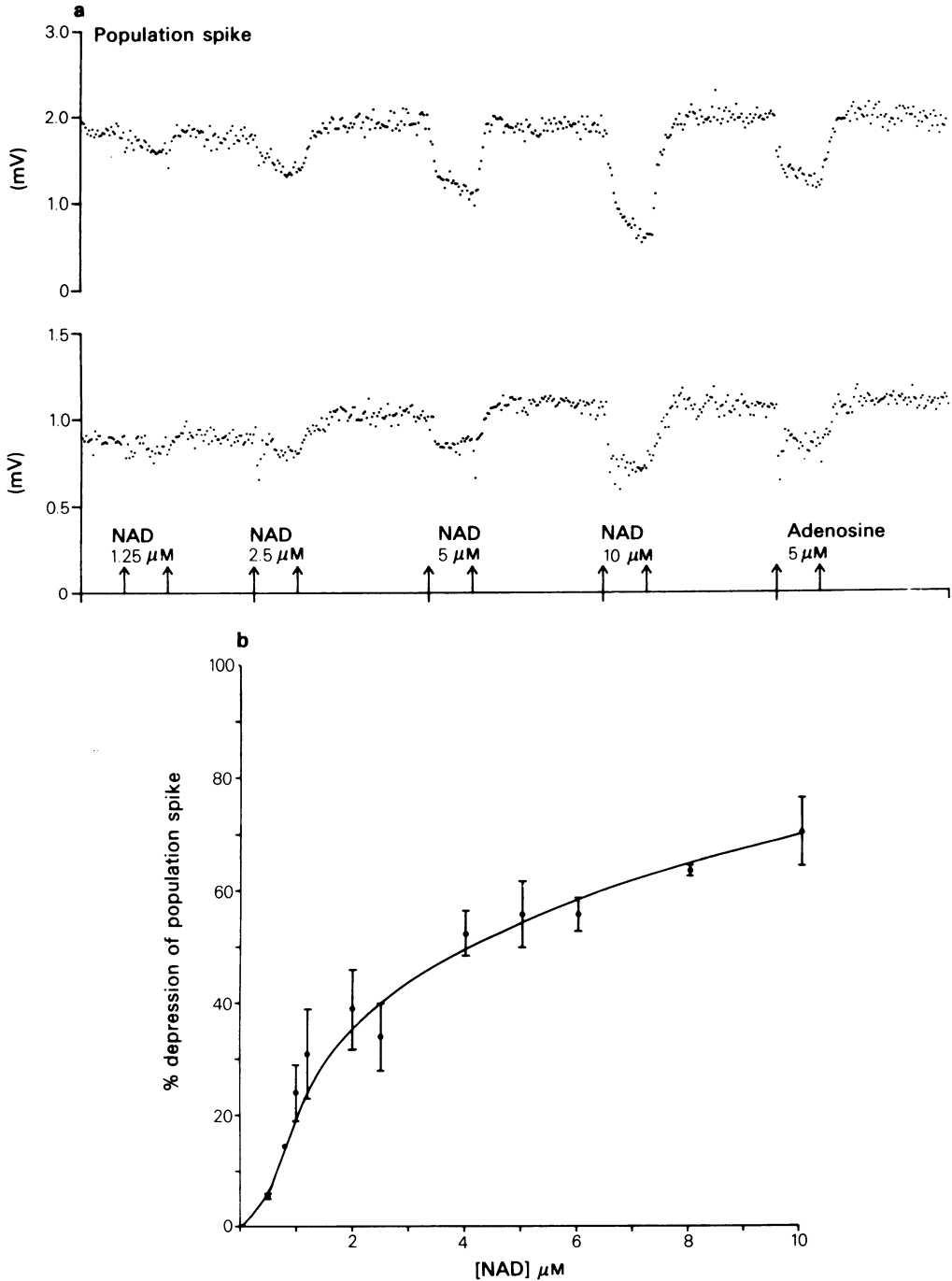


Figure 3 (a) The effect of increasing concentrations of nicotinamide adenine nucleotide (NAD) on the potentials of dentate gyrus evoked by perforant path stimulation. Top panel – amplitude of the population spike; lower panel amplitude of the e.p.s.p. field potential. The final test shows the effect of adenosine 5 μM for comparison. (b) Dose-response curve for the action of NAD on the orthodromic population spike. Each point is mean of $n = 2-12$; s.e.mean indicated by vertical lines.

between the amount bound in the presence and absence of 1×10^{-4} M unlabelled NAD. All assays were conducted in triplicate and the data analysed by the method of Scatchard (1949) to give the equilibrium constant, K_d , and the receptor density, B_{max} . The biphasic Scatchard plots were mathematically analysed to give K_d and B_{max} for the two components using the method described by Burt & Snyder (1975).

Results

Identification of active material

The absorption profile of the effluent from chromatography of the brain extract on Sephadex G15 is shown in Figure 2a. Each major peak was assayed for biological activity as described in Methods. A powerful depressant activity was discovered in the second peak (shaded in Figure 2a). This activity rapidly and reversibly depressed the evoked synaptic potentials but did not block the antidromic invasion of the granule cells (Figure 2b). The active

fraction was then purified by high voltage paper electrophoresis at pH 2.8 and then at pH 6.3, as indicated earlier. The material present in each zone was tested for biological activity and the predominant depressant activity was found in the starting zone in each case. This material was further fractionated on a reverse phase h.p.l.c. column (see Methods) and the effluent from the column collected as 1 ml fractions. The activity was found to run as a single peak on isocratic elution with 2 mM HCl with a retention time of 8 min at a flow rate of 2 ml min^{-1} . The ultraviolet spectrum of this material revealed two absorption maxima at 206 nm and 260 nm. This pattern corresponded closely with that of the dinucleotide, nicotinamide adenine dinucleotide (NAD). In addition, the purified substance had the biochemical properties of NAD and could act as co-factor to glutamate dehydrogenase using glutamic acid as the substrate, an additional absorption at 330 nm developed during the reaction due to the generation of reduced NAD. Final proof was obtained when it was shown that authentic NAD and the active material had identical biological actions on our preparation.

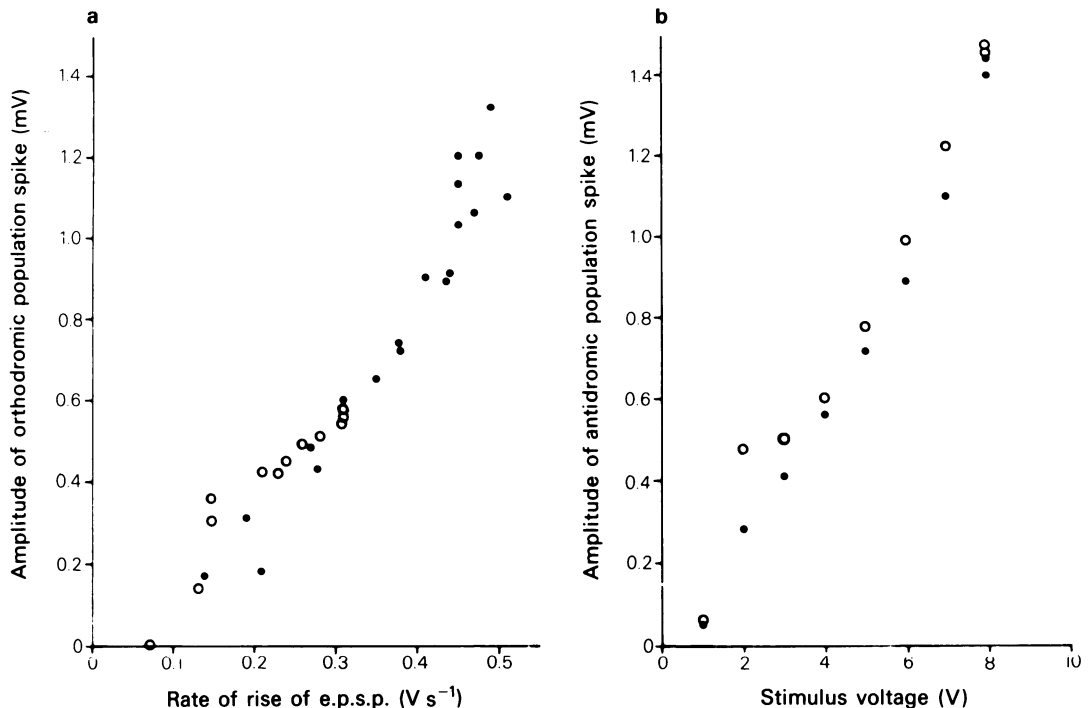


Figure 4 The effect of NAD on granule cell excitability. (a) Relationship between the rate of rise of the population e.p.s.p. and the amplitude of the orthodromic population spike. (b) Relationship between the strength of the stimulus delivered to the mossy fibres and the amplitude of the antidromic population spike. Key: (●) control; (○) + 6 μM NAD in the bathing medium. Note that NAD did not cause either curve to be displaced to the right as a decrease in electrical excitability would require.

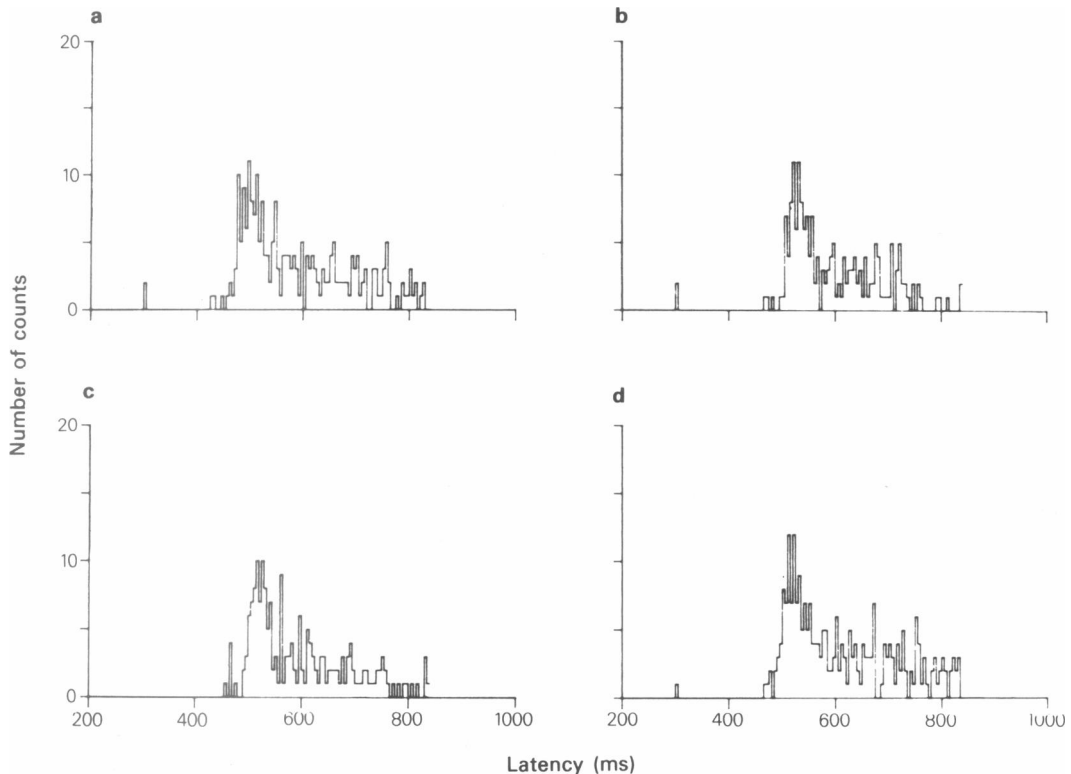


Figure 5 The effect of NAD on the sensitivity of a granule cell to iontophoretically applied glutamate. The results are shown in the form of post stimulus histograms. (a) Control; (b) during the first 1.5 min exposure to NAD $10\ \mu\text{M}$; (c) during exposure to NAD $10\ \mu\text{M}$ for 2–3.5 min; (d) recovery after washing out NAD for 2.5–4 min. The counts at 300 ms in (a), (b) and (d) reflect the switching artifact of the iontophoretic pulse. Stimulus 200 nA L-glutamate for 300 ms every 3 s. Each post stimulus histogram is the result of 20 sweeps.

Site of action of nicotinamide adenine dinucleotide at the synapse

Once the chemical nature of the material responsible for the biological activity had been established, it became possible to examine the site of action of NAD on the synapses in the dentate gyrus and to quantitate its potency. For these experiments, stimuli were delivered to the perforant path before it crossed the hippocampal fissure. The recording electrode was positioned in the granule cell layer of the dentate gyrus under direct visual control some 0.75–1.5 mm from the stimulating electrode. With this arrangement a characteristic field potential is elicited in response to a shock to the perforant path. The response consisted of an initial positive deflection reflecting the depolarization of the dendrites (the population excitatory postsynaptic potential or e.p.s.p.). Superimposed on the e.p.s.p. field potential is a sharp negative deflection reflecting the discharge of the granule cell population and this component is

termed the population spike (see Andersen, Bliss & Skrede, 1971; Lømo, 1971). The amplitudes of both the population e.p.s.p. and the population spike are reduced by NAD and this reduction is dose-related (Figure 3a). In general the population spike was a more sensitive index of the action of NAD and the dose-response curve for the action of NAD on the population spike shown in Figure 3b summarises our data for all preparations. From the curve it can be seen that the ED_{50} for the action of NAD is about $4\ \mu\text{M}$. The error bars indicate that there was considerable variation in the sensitivity of different preparations to the depressant action of NAD. Figure 3a also shows that the potency of NAD is very similar to that of adenosine.

The electrical excitability of the granule cells was tested in four preparations by directly stimulating the mossy fibres (granule cell axons) in the hilus of the dentate gyrus and recording the amplitude of the antidromic population spike. In a further three experiments (two additional preparations) the coupling

between the e.p.s.p. amplitude and that of the population spike was also studied. In no case did NAD ($4\text{--}6\text{ }\mu\text{M}$) depress the electrical excitability of the granule cells (Figure 4a,b).

The action of NAD on the sensitivity of 23 neurones in the granule cell layer to iontophoretically applied L-glutamate and L-aspartate was examined. Twenty-nine tests of the action of NAD on the sensitivity of these neurones to L-glutamate showed little, if any, depressant effect of NAD (18 in the presence of $5\text{ }\mu\text{M}$ NAD, 11 with $10\text{ }\mu\text{M}$ NAD; Figure 5). A further twelve tests with L-aspartate were similarly negative (11 with $5\text{ }\mu\text{M}$ NAD, 1 with $10\text{ }\mu\text{M}$ NAD).

Effect of structural analogues of nicotinamide adenine dinucleotide

Since adenosine is known to depress synaptic transmission in the hippocampus (Dunwiddie & Hoffer, 1980) and the olfactory cortex (Scholfield, 1978) it is possible that the effects we have observed were due to the presence of the adenosine moiety in the dinucleotide. This was confirmed when NADH, NADP

and NADPH were found to be identical in biological effect and potency to NAD. Similarly the α and β forms of NAD had the same potency (Figure 6). These results excluded the possibility that the NAD effect was linked directly to an enzymic process. Nicotinamide mononucleotide was completely inactive (up to $50\text{ }\mu\text{M}$) but adenosine 5' diphosphoribose (NAD less the nicotinamide residue) was equipotent with NAD as was adenosine itself (see Figure 3a). Nicotinamide hypoxanthine dinucleotide was inactive up to $16\text{ }\mu\text{M}$. These results showed that the nicotinamide portion of the molecule was not required for the depressant action but that the adenosine portion was essential.

In agreement with earlier results (Dunwiddie & Hoffer, 1980) theophylline ($4\text{--}20\text{ }\mu\text{M}$) increased the amplitude of the synaptically evoked population spike. This made a proper evaluation of its ability to antagonize the actions of NAD very difficult. Nevertheless, in three preparations this was attempted and a small rightward displacement of the dose-response curve for NAD was observed. The result for a single experiment is shown in Figure 7.

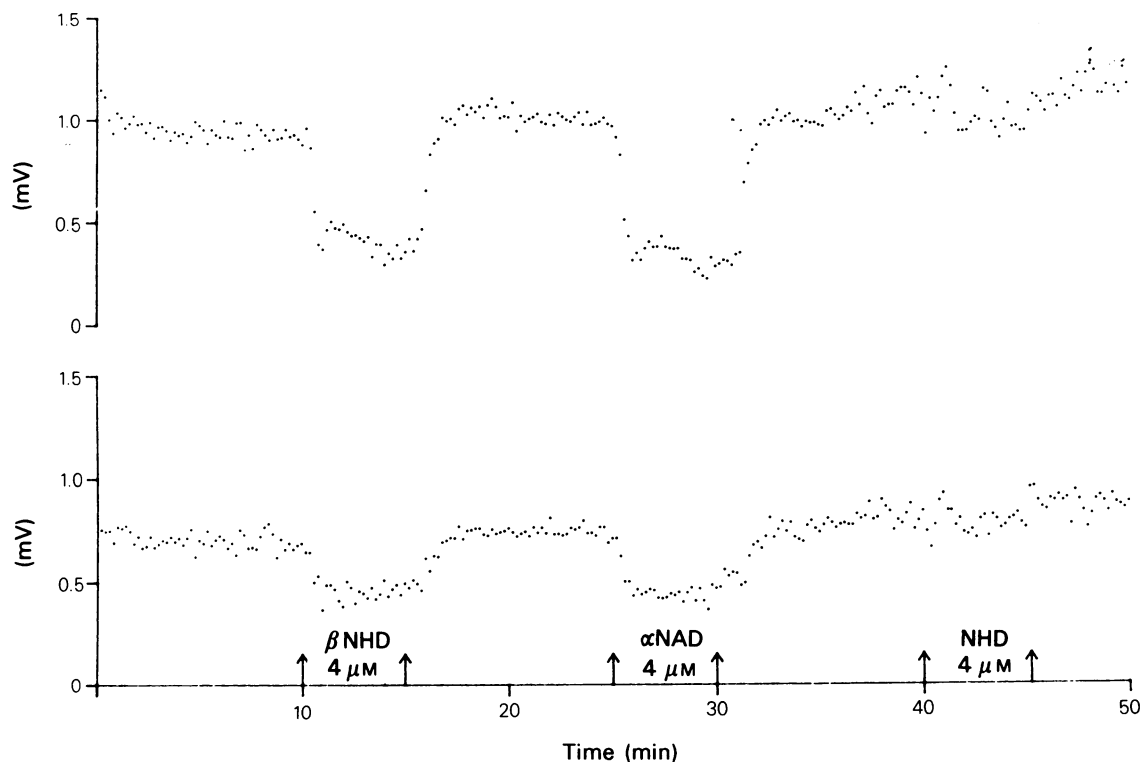


Figure 6 The effect of α and β NAD and nicotinamide hypoxanthine dinucleotide (NHD) on the potentials of the dentate gyrus evoked by perforant path stimulation. Top panel: amplitude of the population spike; lower panel: amplitude of the e.p.s.p. field potential.

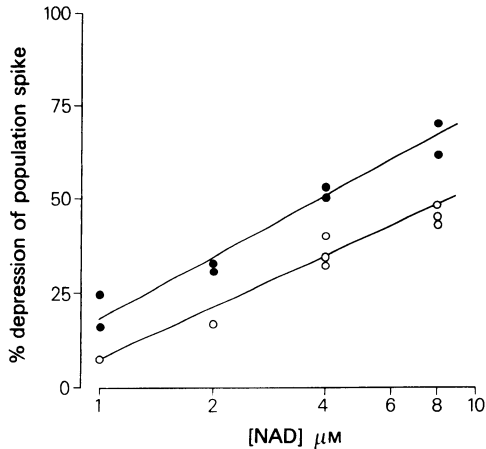


Figure 7 The effect of theophylline ($10\ \mu\text{M}$) on the dose-response relationship for NAD. All results are from a single preparation; each point representing a single test. The lines are log-linear regression lines fitted by the method of least squares. (Note the log axis for the abscissa scale). The ordinate scale is the percentage reduction in the amplitude of the population spike produced by NAD. (●) Control; (○) + $10\ \mu\text{M}$ theophylline.

Characterization of the nicotinamide adenine dinucleotide binding sites

Lysed synaptosomal membranes, free from mitochondria, possess binding sites for $[^3\text{H}]$ -NAD that are saturable (Figure 8b). Scatchard analysis of the displacement of $[^3\text{H}]$ -NAD by increasing concentrations of unlabelled NAD shows two binding sites on synaptosomal membranes (Figure 8a). Binding sites with similar affinity are also present on purified mitochondria. Although the lower affinity site is of similar density on the two organelles, the density of the higher affinity site is five times greater on the synaptosomal membranes (Table 1).

The displacement of $[^3\text{H}]$ -NAD by adenosine 5' diphosphoribose (which differs from NAD only in the absence of a nicotinamide residue) is virtually identical to the displacement produced by unlabelled NAD with IC_{50} s of about $1 \times 10^{-6}\text{M}$ and $1 \times 10^{-4}\text{M}$ for the high and low affinity sites respectively. In contrast, adenosine is unable to displace $[^3\text{H}]$ -NAD fully even at 10^{-3}M and has a distinctly biphasic displacement curve with a lower affinity for the two binding sites; IC_{50} s of about $3 \times 10^{-6}\text{M}$ and 10^{-3}M respectively; see Figure 9.

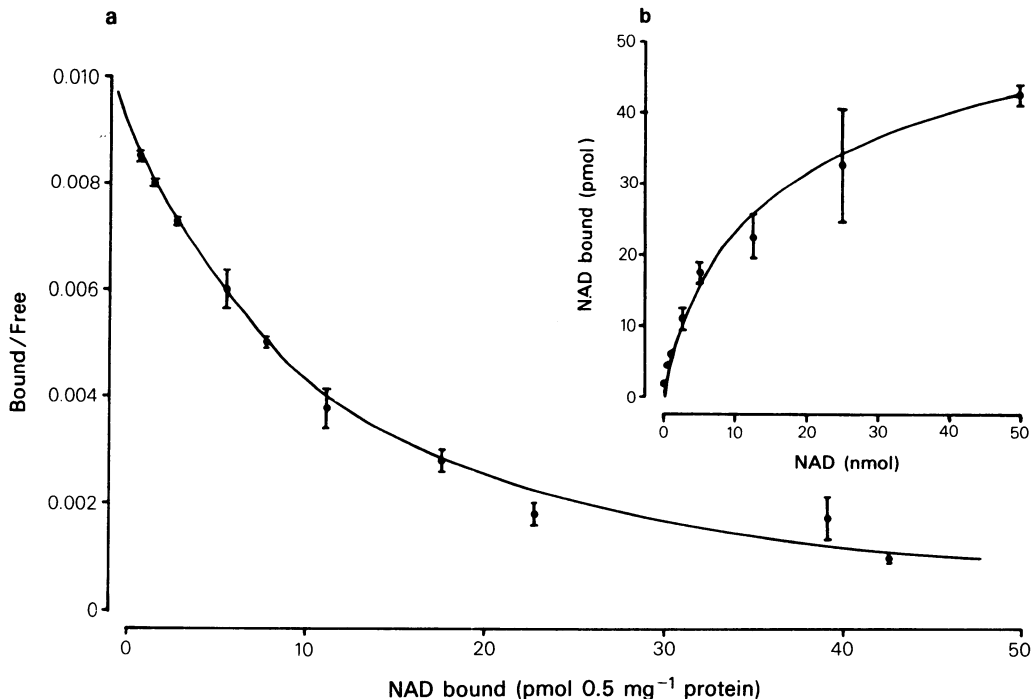


Figure 8 Scatchard Plot and saturation curve for $[^3\text{H}]$ -NAD binding to synaptosomal membranes. (a) Scatchard plot, each point is the mean of three experiments each performed in triplicate; s.e. means are shown. (b) (Inset top right) Saturation curve for specific NAD binding. Each point is the mean of three experiments each performed in triplicate; s.e. means are shown. The abscissa scale shows the amount of NAD in each assay tube. The actual concentrations would be μM (see Methods).

Table 1 Nicotinamide adenine dinucleotide (NAD) binding sites of synaptosomal membranes and mitochondria

Membrane Fractions	High Affinity		Low Affinity	
	K_d (μM)	B_{max} (pmol 0.5 mg^{-1} protein)	K_d (μM)	B_{max} (pmol 0.5 mg^{-1} protein)
Synaptosomal membranes	0.99	5.46	16.8	58.8
Mitochondria	0.56	1.66	14.6	55.8

The data presented are calculated from the slopes and intercepts of the best-fit lines from Scatchard plots of all our binding data for pure synaptosomal membranes and brain mitochondria. The data were analysed by the method of Burt & Snyder (1975) assuming two binding sites. The uncertainty in our estimates for the synaptic membranes can be judged by inspection of Figure 8a. Those for the binding to mitochondria are similar.

Discussion

We have shown that NAD exerts a powerful depressant action on excitatory synaptic transmission in the dentate gyrus. Furthermore, this activity largely accounts for the depressant activity present in extracts of brain tissue that we described earlier (Richards & Snell, 1979). This action of NAD is similar to that reported for adenosine in the hippocampus (Dunwiddie & Hoffer, 1980) and olfactory cortex (Scholfield, 1978). However, it is unlikely that the electrophysiological effects we observe are due to a breakdown of NAD to adenosine as we have found that NAD and adenosine were equipotent (with

ED_{50} s of about $4 \mu\text{M}$) and the onset of the effects observed with NAD was as rapid as that of adenosine. Moreover, preliminary results on the rate of destruction of exogenous NAD by nervous tissue indicates that the greater proportion would not have been broken down within the time to reach equilibrium (30–60 s) (Richards, Snell & Snell, unpublished results). Nevertheless, until more comprehensive data on NAD breakdown are available, we cannot totally exclude the possibility that the action of NAD on synaptic transmission may be due to the production of free adenosine from the dinucleotide.

Since there is little or no spontaneous activity in the slices of hippocampus, the effects of NAD on synaptic transmission in the dentate gyrus must be attributed to direct actions on the perforant path-granule cell system itself and not to effects mediated via other pathways.

In principle, NAD could depress synaptic transmission by blocking impulse conduction in the afferent axons, by suppressing the release of excitatory transmitter substances, by decreasing the sensitivity of the postsynaptic membrane to released transmitters, by increasing the electrical threshold of the postsynaptic cells or by a combination of two or more of these mechanisms. Since we found that NAD did not block the antidromic activation of the granule cells and did not change the stimulus-response characteristics of the granule cell population activated either antidromically or orthodromically (see Figure 4), it is unlikely that NAD acts in this system by decreasing the electrical excitability of neuronal membranes. Therefore it is likely that NAD acts by interfering with some aspect of chemical transmission. Glutamate is thought to be the excitatory transmitter released by the perforant path fibres onto the granule cells (for a review of the evidence see Cotman & Nadler, 1981). However, NAD (5–10 μM) had very little depressant effect on the sensitivity of the neurones in the granule cell layer to iontophoretically applied glutamate or aspartate. This result implies that micromolar concentrations of NAD depress synaptic transmission in the dentate gyrus by reducing the release of transmitter. The possibility that the depressant effects of NAD on potentials are due to interaction with an adenosine receptor cannot be excluded. However, previous studies (Snell & Snell, 1983) would suggest that as NAD has a low affinity for the A2 adenosine receptor such an interaction would be with an A1 adenosine receptor. Nevertheless, we have demonstrated specific and saturable binding sites for NAD on purified synaptosomal membranes. These binding sites appear to be similar to those we have found on mitochondrial membranes (Table 1) although the synaptosomal membranes appear to be some five times richer in the higher affinity binding site. Furthermore the K_d of

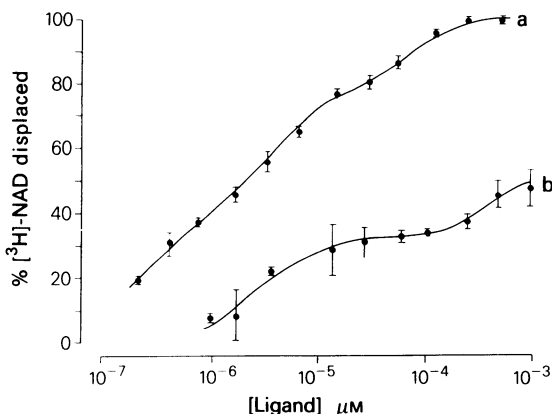


Figure 9 Percentage displacement of specifically bound $[^3\text{H}]$ -NAD ($1.8 \mu\text{M}$) by increasing concentrations of (a) adenosine 5'-diphosphoribose and (b) adenosine. Each point is the mean of the three experiments each conducted in triplicate; s.e. mean are shown.

NAD at the high affinity site was consistent with its potency as a depressant of synaptic activity.

Structure-activity studies of the depressant action of NAD indicate that the nicotinamide portion of the molecule is not required for activity but that the adenosine portion is essential. However, the binding studies show that adenosine itself has a low affinity for these binding sites while the displacement data for adenosine diphosphoribose is virtually identical to that for NAD. It therefore appears that the affinity of the receptor for NAD is due principally to the diphosphoribose portion of the molecule although the adenine residue probably plays an important part in triggering the pharmacological effects of NAD on synaptic transmission. (Nicotinamide hypoxanthine dinucleotide is ineffective).

To summarise, NAD appears to depress excitatory synaptic transmission by binding to specific sites on the presynaptic membrane to cause a reduction in the amount of transmitter released by nerve impulses. These effects of NAD are largely paralleled by adenosine and adenosine mononucleotides and similar results with adenine mono- and dinucleotides

have been obtained in the vas deferens (Stone 1981). Adenosine has been reported to reduce the release of acetylcholine (Vizi & Knoll, 1976) but to be without effect on the sensitivity of cortical neurones to glutamate (Perkins & Stone, 1980). Nevertheless, our results clearly show that NAD and adenosine bind to different sites in the synaptic membranes (see Results) a conclusion that is supported by the differential effects of theophylline on purine release from slices of rat cortex induced by NAD and adenosine (Stone & Perkins, 1981). As whole brain tissue contains about 330 μM NAD (Lolley, Balfour & Samson, 1961), it is possible that small amounts of NAD could be released along with the transmitter to exert a negative feedback control of transmitter output. The effects of theophylline are consistent with this view although the fact that adenosine deaminase also increases the amplitude of hippocampal synaptic potentials (Dunwiddie & Hoffer, 1981) suggests that adenosine may also be directly involved.

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