Inhibition of depolarization-coupled calcium fluxes and transmitter release in vitro by morphine

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Received 17 May 1982

Morphine *Synaptosomes* *Ca^{2+}-flux* *Transmitter release* *Depolarization*

1. INTRODUCTION

There have been many reports of the action of morphine in reducing the release and the turnover of acetylcholine and biogenic amines both in vivo and in vitro [1–5]. Similarly enkephalins have been shown to inhibit the release of acetylcholine and biogenic amines from various in vivo and in vitro preparations [6–10]. These effects are mostly prevented by naloxone.

Release of amino acid neurotransmitters and acetylcholine from the motor cortex of awake, behaviourally normal rats in vivo was suppressed by morphine given intraperitoneally, the effect being prevented by naloxone. This release was evoked either by low concentrations (1 μM) of the depolarizing scorpion venom toxin, tityustoxin delivered onto the cortical surface [11], or by sensory stimulation of the motor cortex via the brachial plexus [12].

The implication is that the actions of morphine reflect the activities and function of a class of endogenous opioids, the enkephalins which serve to modulate neurotransmitter release possibly through an inhibitory action at presynaptic receptors. The inhibitory actions of enkephalins themselves would support this proposal, though caution in interpreting the actions of enkephalin in vitro in terms of a physiological action in vivo have been firmly expressed [13] and some of the published findings have been re-appraised.

Here, the action of morphine on the control and veratrine or high K^+-induced influx of $^{45}Ca^{2+}$ to cerebrocortical synaptosomes in vitro, and its parallel actions on amino acid neurotransmitter release have been investigated.

2. METHODS

Synaptosomes from rat cerebral cortex [14] were incubated at 37°C for 15 min in Ca^{2+}-free Krebs-Tris medium of the following composition (mM): NaCl, 138; KCl, 5; $MgSO_4$, 1.0; Tris, 20 (pH 7.4); glucose, 10; before adding morphine sulphate at the levels indicated. After a further 15 min incubation, veratrine (a depolarizing alkaloid) was added as designated in table 1. Another 10 min incubation was allowed before $^{45}Ca^{2+}$ (Amersham International) was added to give 1.2 mM final conc. and spec. act. 0.8 mCi/mmol. After 10 min the synaptosomes suspensions were added to ice-cold EGTA (final conc. 15 mM) and centrifuged immediately. The supernatants were stored for amino acid analysis whilst the pellets were taken for analysis of $^{45}Ca^{2+}$ content [15]. Amino acids were measured by autoanalysis as in [16].

Potassium was measured by flame photometry using an EEL (Evans Electro Selenium Ltd.) flame photometer.

3. RESULTS AND DISCUSSION

The results in table 1 show that Ca^{2+} influx to the synaptosomes caused by the depolarizing action of veratrine is already maximal at 50 μM. The influx is blocked by tetrodotoxin (1 μM) indicating that the depolarizing action involves Na^+-influx through active-Na^+ channels. Amino acid release is maximal at 75 μM and is similarly blocked by tetrodotoxin.

Morphine, even at 100 μM, was without effect on either $^{45}Ca^{2+}$ influx or on amino acid efflux, evoked by 75 μM veratrine. However, it partially

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75 © 1982 Federation of European Biochemical Societies

Table 1

Effect of morphine on $^{45}Ca^{2+}$-influx and endogenous glutamate release

Veratrine (μM)	75	50	25	K^+ (40 mM)
A: Calcium influx/10 min (nmol/mg protein)				
(No. samples)	(6)	(4)	(4)	(2)
Control (−veratrine, −K+)	3.66 ± 0.13	3.64 ± 0.09	3.71 ± 0.08	3.05
Additions (μM)				
None	7.84 ± 0.10	8.35 ± 0.30	5.72 ± 0.06	4.13
Tetrodotoxin 1	3.73 ± 0.10	–	–	–
Morphine 1	[a] 8.16 ± 0.31	8.33 ± 0.21	5.26 ± 0.18	–
Morphine 5	8.76 ± 0.35	6.58 ± 0.24	5.15 ± 0.28	–
Morphine 15	8.84 ± 0.37	6.44 ± 0.31	4.75 ± 0.22	3.07
Morphine 50	8.41 ± 0.29	6.10 ± 0.37	4.72 ± 0.23	–
Morphine 100	8.24 ± 0.29	5.69 ± 0.23	3.91 ± 0.36	2.98
B: Glutamate release (nmol/100 mg protein)				
No. of samples	(3)	(3)	(3)	
Control – veratrine	321	123	321	–
Additions (μM)				
None	2524	1745	1401	–
Morphine 1	2002	1612	613	–
Morphine 5	1887	1633	280	–
Morphine 15	1954	1489	320	–
Morphine 50	1891	1133	422	–
Morphine 100	1994	1429	325	–

[a] 4 Values (2 expt.) only for morphine added to 75 μM veratrine

Data are mean or mean ± SEM as appropriate for the numbers of samples indicated. Morphine alone (1–100 μM) had no effect on either $^{45}Ca^{2+}$ influx or amino acid release in the absence of veratrine or high K^+

blocked both of these responses to 50 μM veratrine (5–100 μM Ca^{2+}-influx; 15–100 μM, transmitter release). However, $^{45}Ca^{2+}$ influx to the synaptosomes induced by 25 μM veratrine was completely blocked by 100 μM morphine but partially prevented by 1 μM. Influx due to high K^+ (40 mM) was prevented by ≤ 15 μM morphine (table 1). The depolarization induced release of amino acid neurotransmitters, like $^{45}Ca^{2+}$-influx, was also inhibited by morphine though the drug showed a greater potency of action on neurotransmitter release. Thus, for instance, the release evoked by 25 μM veratrine was completely blocked by morphine at 5 μM. Table 1 shows data for glutamate only, but both aspartate and γ-aminobutyrate showed identical behaviour. These inhibitory actions of morphine were all prevented by naloxone at 1 μM (table 2).

Thus, both amino acid neurotransmitter release and $^{45}Ca^{2+}$-influx to synaptosomes can be reduced or blocked by morphine over 1–100 μM provided the depolarizing stimulus is not too great and this

Table 2

Effect of naloxone on morphine-inhibition of calcium fluxes and transmitter release

Additions	μM	$^{45}Ca^{2+}$ influx (nmol/mg protein)	Glutamate release (nmol/100 mg protein)
None	–	3.45	289
Veratrine	25	5.72[a]	1407
Veratrine	25	5.15[a]	280
Morphine	5		
Veratrine	25	5.65	1025
Morphine	5		
Naloxone	1		
Veratrine	25	3.91[a]	325
Morphine	100		
Veratrine	25	5.75	1032
Morphine	100		
Naloxone	1		

Data are mean of 3 or 4 ([a]) values. Naloxone was added 10 min before addition of morphine. Other conditions are as for table 1

effect is blocked by naloxone. The greater potency of action of morphine on transmitter release compared with $^{45}Ca^{2+}$-influx is presumably due to an exponential component in the mechanisms whereby Ca^{2+} initiates transmitter release, i.e., a small decrease in $^{45}Ca^{2+}$-influx would be reflected as a much larger decrease in transmitter release. Equally, only a fraction of the $^{45}Ca^{2+}$ entering may be responsible for transmitter release.

Whether the concentrations of morphine employed here are relevant to those reached in vivo, and form part of the primary mechanism of action of morphine (and, by implication of the enkephalins) remains uncertain [13]. There is also the question of whether morphine acts by causing the blockage of both Ca^{2+} influx and transmitter release independently by an action on a third process. For instance, if morphine were to prevent depolarization per se, the same 2 effects would be seen to occur simultaneously. This possibility was examined. Measurement showed (table 3) that the substantial release of K^+ to the incubation medium caused by veratrine was not prevented by morphine even at the highest levels used (100 μM). This suggests that veratrine did induce its usual degree of depolarization in the presence of morphine, and the latter's primary action was rather to reduce or prevent Ca^{2+} influx, and thereby modulate neurotransmitter release. A primary action of enkephalins on the Ca^{2+} current associated with the action potential has been reported [17,18].

Table 3

Effect of veratrine and morphine on K^+-levels of synaptosomes

Additions	μM	K^+ content (μ equiv./100 mg protein)
None	–	30.7 ± 0.19
Veratrine	25	19.0 ± 0.06
Veratrine	50	18.0 ± 0.12
Veratrine	50	18.7 ± 0.46
Morphine	1	
Veratrine	50	17.7 ± 0.54
Morphine	15	
Veratrine	50	18.0 ± 0.39
Morphine	100	
Veratrine	25	22.3 ± 0.24
Morphine	1	
Veratrine	25	19.8 ± 0.26
Morphine	15	
Veratrine	25	20.8 ± 0.08
Morphine	100	

Cortical synaptosomes were incubated at 37°C as in section 2. Values are mean ± SEM for 4 values from 2 synaptosome preparations

ACKNOWLEDGEMENTS

D.D. was an MRC postgraduate student. The work was supported by an MRC programme grant.

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