Inhibition by morphine of the release of [^{14}C]acetylcholine from rat brain cortex slices

Morphine elevates levels of total brain acetylcholine in rats and mice (Giarman & Pepeu, 1962; Hano, Kaneto & others, 1964). In the rat brain, "bound" acetylcholine was increased while "free" acetylcholine was decreased (Crossland & Slater, 1968). Previously morphine had been shown to decrease the release of acetylcholine from guinea-pig ileum (Schaumann, 1957; Paton, 1957; Cox & Weinstock, 1966) and from brains of anaesthetized cats (Beleslin & Polak, 1965; Beleslin, Polak & Sproull, 1965).

KCl accelerates the release of acetylcholine; for example, from rat isolated diaphragm (Mitchell & Silver, 1963), rat cerebral cortex slices (Polak & Meeuws, 1966) and the superior cervical ganglion of the cat (Brown & Feldberg, 1936).

The effect of morphine on the accelerated release of [^{14}C]acetylcholine by KCl from rat brain cortex slices has now been examined.

Slices of rat cerebral cortex were prepared as described by McIlwain & Rodnight (1962). Two incubation media were used: the first contained (M) 0·13 NaCl, 0·004 KCl, 0·002 $CaCl_2$, 0·025 $NaHCO_3$, 2×10^{-4} eserine sulphate (Mann, Tennenbaum & Quastel, 1939) and will be referred to as "4 mM KCl medium"; the second contained (M) 0·103 NaCl, 0·031 KCl, 0·002 $CaCl_2$, 0·025 $NaHCO_3$, 2×10^{-4} eserine sulphate (Mann & others, 1939) and is designated "31 mM KCl medium". Both media contained 0·005 M [^{14}C]glucose (uniformly labelled) except when employed for reincubation or for extraction of [^{14}C]acetylcholine from the slices. Media were adjusted to pH 7·4 with HCl before incubation. Slices (200 ± 10 mg wet weight) were placed in 50 ml beakers containing 3 ml of incubation medium. Vessels were incubated at 37° for varying periods of time in an atmosphere of 95% oxygen and 5% carbon dioxide.

In the first set of experiments, slices were incubated in 31 mM KCl medium with and without 10^{-3} M morphine for 75 min. In the second experiments slices were first incubated in 4 mM KCl medium for 60 min to accumulate [^{14}C]acetylcholine in the slices; one aliquot of these slices was reincubated in 31 mM KCl medium for 15 min, while a second portion was reincubated in 31 mM KCl containing morphine 10^{-3} M for the same period.

At the end of the incubations, the vessels were chilled to 0° in crushed ice. Slices were separated from media by centrifugation at 2200 *g* for 20 min at 0°. Slices were then homogenized in 3 ml of fresh incubation medium. Both slices and media were analysed for their [^{14}C]acetylcholine content as described by Browning & Schulman (1968).

Results in Table 1 show that when morphine (10^{-3} M) was present in the incubation

Table 1. *Influence of morphine on the formation or release of* [^{14}C]*acetylcholine by slices of rat cerebral cortex*

	^{14}C-Acetylcholine μg/g				
	Formation			Release	
Addition	Total	Slices	Medium	Slices	Medium
None	18·10 ± 0·74	2·30 ± 0·16	15·70 ± 0·73	1·08 ± 0·05	1·12 ± 0·15
Morphine 10^{-3} M ..	12·25 ± 0·90*	1·99 ± 0·07	10·30 ± 0·69*	1·23 ± 0·04†	0·75 ± 0·04†

* $P < 0·01$ † $P < 0·05$.

medium there was a decrease in the amount of [^{14}C]acetylcholine formed but this occurred largely in the medium. Since the [^{14}C]acetylcholine in the medium was synthesized in the slices and transferred to the medium as a result of the releasing action of KCl, it may be presumed that morphine retarded this action of KCl. That this may be so is seen from the effect of morphine on the release of preformed [^{14}C]acetylcholine from slices. The presence of morphine in the incubation medium was associated with a significant decrease in the amount of [^{14}C]acetylcholine released from the slices into the medium. And, conversely, the amount of [^{14}C]acetylcholine that remained in slices was greater in the incubation medium containing morphine.

Morphine inhibits the release of acetylcholine in other systems, for example, from guinea-pig ileum (Schauman, 1957; Paton, 1957; Cox & Weinstock, 1966) and from cat brain (Beleslin & Polak, 1965; Beleslin & others, 1965). Our experiments substantiate these reports and indicate that morphine had a similar effect on rat cerebral cortex. The increased acetylcholine content of rat brain after administration of certain drugs has been attributed to inhibition of acetylcholine release by these drugs rather than to stimulation of acetylcholine synthesis or to inhibition of cholinesterase (Crossland & Merrick, 1954). Our finding that morphine inhibited the release of acetylcholine from rat cerebral cortext suggests that the observed increase in total and "bound" acetylcholine content of rat brain associated with morphine administration (Giarman & Pepeu, 1962; Crossland & Slater, 1968) may well be due to inhibition of acetylcholine release at cholinergic synapses in the brain. This effect would also explain the decrease of "free" acetylcholine observed by Crossland & Slater (1968) in brain after morphine administration to rats.

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