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EFFECT OF MORPHINE DEPENDENCE AND WITHDRAWAL ON BRAIN ACETYLCHOLINE CONTENT AND RELEASE

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INTRODUCTION

A number of drugs alter the acetylcholine content of the brain. We turned our attention to morphine, with the aim of learning more about the processes that lead to physical dependence. There is evidence both from our own work (Crossland et al., 1984) and that of others (Beleslin D. et al., 1965 and Beleslin D. et al., 1965) that morphine depresses the release of acetylcholine in the brain. If this means that morphine inhibits activity in the central nervous system as a result of its being able to suppress the release of transmitter from cholinergic neurones, it may be that morphine tolerance occurs when compensatory mechanisms re- establish a normal rate of acetylcholine release notwithstanding the restraint imposed by the morphine. In such a situation the sudden removal of this restraint by withdrawal of the drugs, might be expected to lead to large "rebound" release of acetylcholine that would continue until the acetylcholine releasing mechanisms returned to their normal level of activity. Our experiment support the idea that morphine abstinence syndrome is due to a "flooding" of the brain with acetylcholine in this way.

MATERIALS AND METHODS

Production of Morphine Dependence: Animals (female Wistar strain albino rats of body weight 100 to 120g and female C.D. mice of body weight 25 to 35g) were housed singly and given unrestricted access to food. Their drinking fluid, delivered from graduated bottles, consisted of 45% solution of sucrose containing morphine hydrochloride at an initial concentration of 1mg/ml. This was maintained for one week and was then increased at the rate of 1 mg/ml each week for another four weeks. Control animals received syrup only. Food and fluid intakes were measured daily.

The withdrawal syndrome was precipitated by i.p. administration of either naloxone hydrochloride (4 mg/kg) or nalorphine (150 mg/kg) to animals habitated to morphine.

In some experiments, those involving measurement of acetylcholine release from the cortex (Crossland J et al., 1984) the habituated animals were anesthetized with 'Dial' compound (0.8 ml/kg by i.p.) before being given naloxone or nalorphine. The intensity of the withdrawal syndrome was assessed by a method that was developed by Fuentes (1970) and Hunt (1971).

'Free' and 'bound' acetylcholine was extracted from the brain samples by the method of Crossland and Slater (1968) Acetylcholine was assayed either on the isolated dorsal muscle of the leech or on the isolated rectus abdominis muscle of the frog, depending on the amount of acetylcholine available for assay.

RESULTS

Results obtained by this study are shown in Table-I. This shows that, although the 'free' and 'bound' acetylcholine in the brains of morphine habituated animals was no different from that in untreated animals, the 'free' acetylcholine of brain underwent a massive increases (216 percent in rats and 322 percent in mice) at the time when the withdrawal syndrome induced by naloxone was at its height. A second dose of naloxone given to habituated rats two hours after the first (when the original syndrome had subsided) induced a very mild additional syndrome (the intensity score averaged no more than 2.4) and correspondingly smaller increase in the 'free' acetylcholine (Table-II). An abstinence syndrome was successfully produced in Dial-anesthetized rats but both the intensity of the syndrome (the score of which averaged 3.8) and the associated rise in the 'free' acetylcholine content of the brain (77 percent) were considerably smaller than those in unanesthetized animals (Table-III).

Figure 1 and 2 illustrate the changes in the release of acetylcholine from the cortices of habituated rats challenged by naloxone. It is clear that in a general way, these changes mirror the changes in 'free' acetylcholine content of brain. In Figure- 2, the relatively trivial effect of a second dose of naloxone in quite evident. To establish, in a rigidly quantitative way, the link between acetylcholine release and the 'free' acetylcholine content of the cortex it was necessary to make determinations of the 'free' acetylcholine in brains from which acetylcholine release was also being measured. When this was done, high and highly significant correlations were found between acetylcholine release and the 'free' acetylcholine content both of the cortex immediately underneath the area from which acetylcholine was being collected ($X = +0.915$, $n+52$, $P < 0.001$) and the rest of the brain ($X = 0.909$, $n+52$, $P < 0.001$). The individual values that gave rise to the first mentioned of these correlations are shown in Figure-3, from which it is evident that the correlation holds good irrespective of whether the animals were habituated to morphine, in a normal state or suffering a withdrawal syndrome.

There were rather low and negative (but still significant) correlations ($r \approx 0.4$, $P \approx 0.2$) between the rate of release of acetylcholine and the contents of 'bound' acetylcholine both in the cortex and in the rest of the brain.

DISCUSSION

The results reported in this paper confirm the validity of the view, not previously tested by direct experiment, that the brain content of 'free' acetylcholine as defined by Crossland and Slater (1968) mirrors the rates of acetylcholine release by the brain, at least when these changes in content are brought about by morphine and related compounds. Before the discovery of the endogenous opoids and their receptors it was believed by many that morphine exerted its actions by way of acetylcholine receptors. While we now have to accept that morphine and its congeners act on the opoid receptors, it seems likely that excitation of these receptors has an affection cholinergic neurones and that this effect is widespread.

Several other groups have produced evidence that morphine inhibits the release of acetylcholine from the brain (Beleslin D. et al. (1965); Beleslin D. et al. (1965); Casamenti F. et al. (1980); Coutinho-Netto J. et al. (1982); Jhamandas K. et al. (1977). Other have indicated that the enkephalins also modify the release of acetylcholine from the central nervous system (Jhamandas K. et al., 1970).

It seems, from the experiments reported here, that acetylcholine in the brains of mice suffering a morphine abstinence syndrome undergoes changes as dramatic as those seen in the rats. This finding is difficult to reconcile with the views of way and his colleagues, who, over a period of years have maintained (Way E.L. et al., (1968) that 5-hydrotryptamine and not acetylcholine is intimately involved in the production of the morphine abstinence syndrome in mice.

All the experiments conducted in this study seem to support the idea that cholinergic neurones are widespread throughout the brain and that acetylcholine is involved in many central nervous phenomena.

Table I

The effect of naloxone on the 'free' and 'bound' acetylcholine contents of the brains of rats and mice habituated to morphine for four weeks

A. RATS Habituated to morphine for 4 weeks and given:	Acetylcholine contents			
	'free' ug/g	'bound' ug/g	'total' ug/g	free/total %
Saline	0.31 ± 0.03	2.12 ± 0.27	2.43 ± 0.30	12.8 ± 0.66
Nalosone	0.98 ± 0.06	2.03 ± 0.36	3.02 ± 0.30	34.3 ± 5.6
Percentage change	+ 216.1	-4.2	+ 24.2	+ 167.5
t	11.6	0.2	1.28	3.7
Significance(P)	< 0.001	NS	NS	< 0.01
B. MICE Habituated to morphine for 4 weeks and given:				
Saline	0.23 ± 0.07	2.40 ± 0.17	2.63 ± 0.18	8.74 ± 0.34
Naloxone	0.97 ± 0.06	2.22 ± 0.19	3.19 ± 0.21	30.4 ± 8.09
Percentage change	+ 321.7	- 7.5	+ 21.2	+ 247.8
t	8.1	0.72	2.07	2.48
Significance(P)	0.001	NS	NS	< 0.05

Each value is the mean ± standard error of observations made on 4 animals. Animals were killed 15 minutes after the intra-peritoneal administration of naloxone, 4 mg/kg.

Table II

The effect of a second dose of naloxone on the acetylcholine contents of habituated rats

Habituated animals	Acetylcholine contents			
	'free' ug/g	'bound' ug/g	'total' ug/g	free/total %
4 hours after Ist dose of naloxone	0.31 ± 0.05	2.12 ± 0.27	2.43 ± 0.30	10.80 ± 0.00
15 minutes after 2nd dose of naloxone	0.46 ± 0.02	2.05 ± 0.10	2.51 ± 0.11	18.30 ± 1.02
Percentage change	+ 48.3	- 3.3	+ 3.2	+ 43.0
t	5.0	0.25	0.25	4.5
Significance(P)	< 0.01	NS	NS	< 0.01

Each value is the mean ± standard error of observations made on 4 animals. The second dose of naloxone (4 mg/kg) was given by intraperitoneal injection 4 hours after the first dose and the animals were killed 15 minutes after receiving their second dose.

Table III

The effect of naloxone and an additional dose of morphine on the 'free' and 'bound' acetylcholine contents of the brains of annesthetized rats habituated to morphine

Injection	Acetylcholine contents			
	'free' ug/g	'bound' ug/g	'total' ug/g	free/total %
Saline solution	0.60 ± 0.05	2.24 ± 0.31	2.85 ± 0.31	22.2 ± 4.03
Naloxone	1.06 ± 0.03	2.06 ± 0.27	3.12 ± 0.26	34.8 ± 3.5
Percentage change	+ 76.6	- 14.8	+ 9.4	+ 56.4
t	12.0	0.87	0.67	2.34
Significance(P)	< 0.001	NS	NS	NS

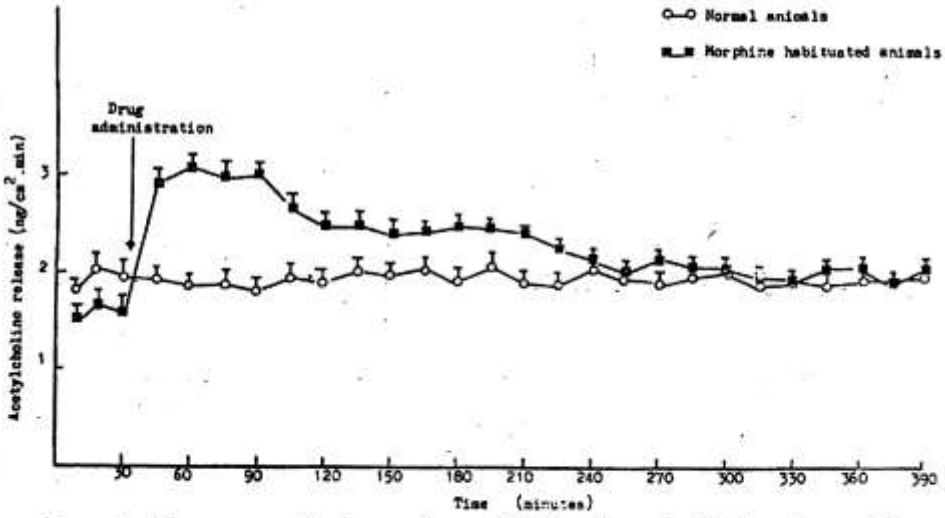

Figure 1: The course of release of acetylcholine from the brains of morphine habituated rats given naloxone. Each point gives the mean and standard error of values from four brain.

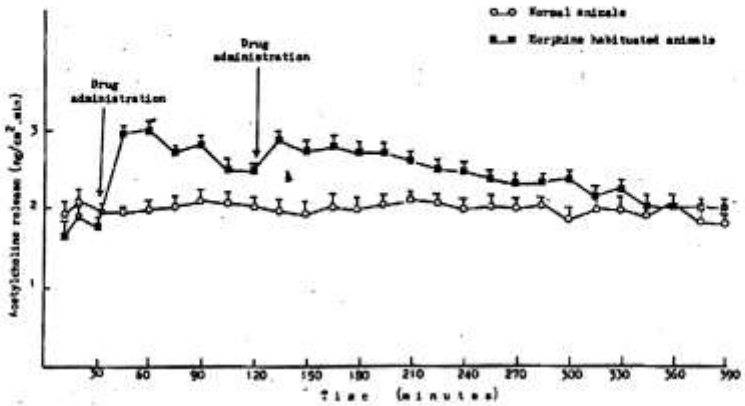

Figure 2: The effect of a second dose of naloxone on the acetylcholine out put from the brains of rats habituated to morphine. Each point gives the mean and standard error of values from four brain.

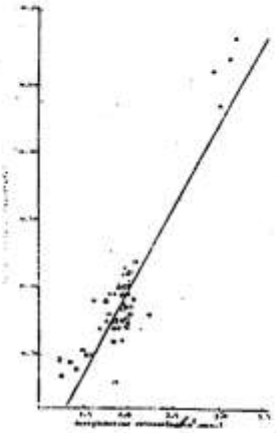

Figure 3: Correlation between acetylcholine release and the 'free' acetylcholine content of the cortex.

Key:

- -- **Untreated animals (8)**
- -- **Untreated animals given naloxone, 4mg 1kg (12)**
- -- **Normal animals given nalorphine, 100 mg/kg (4)**
- -- **Normal animals given morphine, 30, 100 mg/kg (8)**
- -- **Animals habituated to morphine (8)**
- -- **Habituated anials given morphine, 100 mg/kg (4)**
- -- **Habituated animals given naloxone, 4 mg/kg (4)**
- -- **Habituated animals given two successive doses of naloxone, 4 mg/kg (4)**

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