Blood Citric Acid Cycle and Individual Response to Morphine Analgesia in Rats[1]

The effect of morphine on glycolytic intermediaries has been studied by numerous investigators[2-5]. The significant effects on glycolytic intermediaries suggest the possibility of a correlation between glycolytic activity and analgesia. The purpose of this study was to determine whether existing blood levels of citric acid cycle intermediaries were related to the effectiveness of morphine as an analgesic.

Two studies were performed, one to determine whether a relationship between blood levels and individual, response to morphine existed, and the second to determine whether this relationship was due to differential uptake of morphine or to biochemical factors.

Methods. Experiment 1. 11 male Holtzman rats were used. Sensitivity to pain was measured using the KAYAN et al.[6] modification of the method of JÓHANNESSON and WOODS[7]. The animal was dropped into a 6 inch plastic cylinder which was placed on a 55 °C hot plate and a plastic top was placed on top of the cylinder. Animals remained on the plate until either a vigorous licking of any paw or an attempt to jump out of the cylinder occurred. As soon as either criterion response had been performed, the animal was removed from the plate. The time between placement on the hot plate and performance of either criterion response was recorded, and was designated as pre-drug reaction time. This measure provided the baseline for future comparisons. At this time, the animal was injected with morphine sulfate (2.5 mg/kg as free base) subcutaneously in the midline of the back and returned to a home cage.

Thirty minutes after injection, each animal was again dropped onto the hot plate and reaction time (time between placement on the plate and performance of either criterion response) was again measured. The difference between pre-morphine reaction time and this post-morphine reaction time is referred to as analgesia.

Before testing sensitivity to pain, each animal was placed in a restrainer and the tip of the tail cut. 50 µl of blood was drawn as rapidly as possible into a capillary pipet and placed into 2 ml of acetone/HCl (100:3 V:V). 5 h of extraction at room temperature were allowed. The acetone/HCl extraction medium was transferred along with all tissue to centrifuge tubes and centrifuged for 20 min at 2000 rpm. The acetone was decanted and the tissue resuspended in 1 ml of acetone.

The wash was allowed to stand in the stoppered centrifuge tube for 30 min, recentrifuged, and added to the original extraction medium. All 3 ml of acetone were air-dried employing a cold air stream. When dry, 2 ml of anhydrous methanol/HCl was added and the sample was esterified for 40 min in an 85 °C oven.

A five µl sample was analyzed with a 6 feet glass U tube packed with 5% FFAP (Varian Associates). A Bendix model 2500 gas chromatograph was used with the following conditions: injector 210 °C, detector 240 °C, column 80 °C for 2 min, then increased to 200 °C at 8 °C/min, nitrogen flow at 40 ml/min. In this manner, peripheral blood levels of pyruvate, lactate, fumarate, succinate, malate, α-ketoglutarate, and citrate were determined.

Experiment 2. Analgesic testing procedure: The procedure for this replication was the same as in the first study, except that 20 animals were used.

Analytical Procedure. Immediately after analgesia testing, the animal was decapitated and the brain removed over ice. Whole-brain morphine levels were determined by the method of FINKLE et al.[8], except that the extract was not boiled. The extraction and derivatization of the Krebs cycle acids was the same as in the previous study. In this study, methyl esters were chromatographed once on an SE-30 column under the following conditions: detector 260 °C, injector 210 °C, column 150 °C isothermal for 3 min, then increased to 255 °C at 4 °C/min.

Statistical analysis. In Experiment 1, ϱ correlations[9] were calculated between 1. rank on substrate level and rank on pre-drug reaction time and 2. rank on substrate level and rank on analgesia (difference between pre-drug and post-drug reaction times).

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Table I. Average substrate levels (in mg/100 ml ± S.E.) and ϱ correlations between substrate levels and pre- and post-drug reaction times

Substrate	Average level	ϱ Correlations	
		pre-drug	post-drug
Pyruvate	1.05 ± 0.1	− 0.24	0.84[a]
Lactate	10.7 ± 0.9	− 0.04	0.51[b]
Fumarate	1.27 ± 0.1	− 0.19	0.37
Succinate	7.21 ± 0.5	− 0.12	0.04
Malate	5.02 ± 0.3	− 0.01	0.08
α-Ketoglutarate	2.16 ± 0.2	0.09	0.19
Citrate	3.51 ± 0.4	0.17	0.40

[a] $p < 0.01$. [b] $p < 0.05$.

Table II. Average substrate levels (in mg/100 ml ± S.E.) and ϱ correlations between substrate levels, pre- and post-drug reaction time, and brain morphine level

Substrate	Average level	ϱ Correlations		
		pre-drug	post-drug	Morphine levels
Pyruvate	1.02 ± 0.1	− 0.17	0.80[a]	0.35
Lactate	10.9 ± 1.0	0.05	0.45[b]	0.32
Fumarate	1.20 ± 0.2	− 0.21	0.39	0.34
Succinate	7.11 ± 0.6	0.04	0.08	0.23
Malate	4.96 ± 0.4	− 0.10	0.04	0.64[a]
α-Ketoglutarate	2.21 ± 0.2	0.13	0.32	0.55[b]
Citrate	3.60 ± 0.5	0.21	0.60[b]	0.26

[a] $p < 0.01$. [b] $p < 0.05$.

In Experiment 2, ϱ correlations were calculated between 1. rank on substrate level and rank on analgesia, 2. rank on substrate level and rank on brain morphine level and 3. rank on analgesia and rank on brain morphine level.

Results. Experiment 1. The ϱ correlations are shown in Table 1. In addition, average blood levels for each substrate are shown. These levels are similar to literature values[10]. As can be seen, blood levels of pyruvate and lactate correlated with analgesia, while no substrate correlated with pre-drug reaction time.

Experiment 2. Table II shows that pyruvate and lactate, as well as citrate levels correlated with analgesia. Only malate and α-ketoglutarate correlated with whole-brain morphine levels. The average brain level of morphine is also shown in Table II. The correlation between whole brain morphine level and analgesia was $+0.47$ ($p < .05$). The average brain level of morphine was 0.06 ± 0.005 µg/g (S. E.).

Discussion. The finding of a positive correlation between blood pyruvate, lactate, and possibly citrate with analgesia suggests that high glycolytic flux (as measured by high pyruvate levels) may be a component of analgesia. Since there were no significant correlations between substrate levels and pre-drug reaction time, it appears unlikely that peripheral intermediary levels are associated with basic sensitivity to pain. High levels of malate and α-ketoglutarate were associated with high levels of brain morphine, suggesting the these substrates may be important in morphine uptake.

The finding of a relatively low (0.47) but statistically significant correlation between brain morhpine level and analgesia is in agreement with the findings of MULÉ et al.[11] and MULÉ[12]. This suggests that the intensity of the metabolic change produced by morphine may be more important in determining the level of analgesia than the amount of drug present.

The data also suggest that some of the metabolic changes involved in morphine-induced analgesia may be mediated through the citric acid cycle.

Zusammenfassung. Es konnte gezeigt werden, dass bei Ratten eine Beziehung besteht zwischen der analgetischen Wirkung von Morphin und den Blut-Konzentrationen einiger Metaboliten des Krebs-Zyklus.

A. D. SHERMAN and C. L. MITCHELL

Department of Pharmacology,
College of Medicine,
University of Iowa,
Iowa City (Iowa 52240, USA), 3 July 1972

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Selective Suppression of Rapid Eye Movement Sleep (REM) by Fusaric Acid, an Inhibitor of Dopamine-β-Oxidase

Several converging lines of evidence point to an important participation of noradrenaline (NA) in the production of REM. However, existing data are still conflicting. In the cat, lesioning of the locus coeruleus rich in the NA-containing neurons[1] has been reported by some[2] to suppress REM selectively, while others[3] have been unable to confirm this finding. Experiments performed with inhibitors of enzymes at different steps of biosynthesis of NA have shown that decreased concentration of NA in the brain leads to reduction in the amount of REM[4,5]. This is in line with the fact that administration of DOPA restores reserpine-suppressed REM[6]. However, inhibition of tyrosine hydroxylase by α-methyl-*p*-tyrosine has been shown to increase the REM amount, while decreasing the concentration of NA in the brain[7].

It seems that, with respect to the relationship between NA and REM, conflicts among the results obtained through pharmacological interventions may arise from coincidental involvement of factors other than NA. For this reason we have been prompted to study the effect on REM of fusaric acid (5-butylpicolinic acid), which possesses a very pertinent property without unfavorable complications. This drug has no reported action other than inhibition of dopamine-β-oxidase and consequent reduction of the concentration of NA in the central nervous system as well as in the periphery[8].

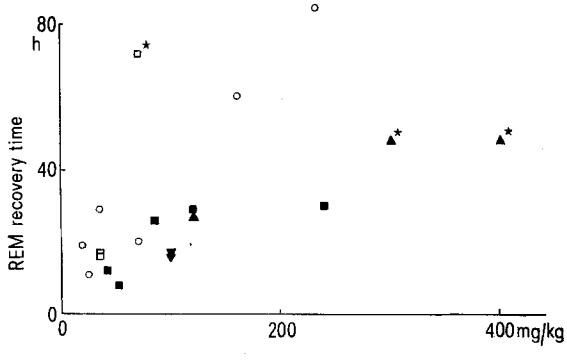

Fig. 1. REM recovery time after single administrations of fusaric acid or its calcium salt in 4 cats. Ordinate; time in h for the cumulative amount of REM to catch up to the control level after drug administration. Abscissa; the amount of drug administered (open marks, peritoneal injection of fusaric acid; filled marks, oral administration of the calcium salt). Marks accompanied with asterisk indicate failure to catch up to the control level within the follow-up period.

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