Enhancement of the Analgesic Effect of Morphine by Sodium Diethyldithiocarbamate in Rats

From the observation that morphine decreased the catecholamine (CA) content in the brain of certain species of animal[1-3], many authors have suggested that manifestation of the analgesic action of morphine might be related to the CA level in the brain of these animals. Thus, the antagonistic action of reserpine on morphine analgesia, first described by SCHNEIDER[4], has been widely examined and it is believed that depletion of the brain biogenic amines caused by reserpine is responsible for the antagonism[5]. However, it is uncertain from data with reserpine which are the chief amines involved in the analgesic action of morphine, because reserpine reduces the levels of noradrenaline (NA), dopamine (DA) and serotonin.

Sodium diethyldithiocarbamate (DDC), which lowers the level of NA in rat brain by inhibiting DA-β-hydroxylase[6], thus seems a very suitable drug to use in solution of this problem. This paper describes the effect of DDC on the analgesic action of morphine in relation to the NA and DA contents of rat brain.

Methods. Male Sprague-Dawley rats, weighing 130 to 180 g, were used. Analgesia was estimated by the tail clip method modified by TAKAGI et al.[7]. The pressure for rats was found to be ca. 4 kg, using a clip of 6 mm diameter. To avoid possible damage of the tail, the clip was removed after 15 sec, and if the animal did not respond within this time, a score of '15 sec' was given. In the hot plate method[8], if the animals did not respond by licking the hind legs or jumping from the bath, in 60 sec, they were removed from the plate and given a score of '60 sec'. After administration of morphine, the response was estimated at 15 min intervals for about 180 min in case of tail clip method and 120 min in hot plate method. With both methods, experiments were performed in the morning.

For determination of CA, animals were sacrificed by dipping them into dry-ice acetone solution and CA was extracted from the brain as described previously[9]. NA was determined by the method of EULER and FLODING[10] and DA by that of LAVERTY and TAYLOR[11].

Results. As shown in Table I, 4 h after the second injection of DDC (500 mg/kg × 2, s.c.), the analgesic action of 10 mg/kg of morphine administered s.c. was potentiated. Morphine analgesia was also enhanced, 1 h after a small dose of DDC (350 mg/kg, i.p.). The same results were obtained with the hot plate method (Table II). Sometimes, on treatment with DDC, the normal response time was slightly prolonged with tail clip method, but these were rarely observed with hot plate method. 4 h after the second dose of DDC, there was a marked decrease in the NA level in the brain, whereas the DA content did not change significantly (Table III). 1 h after a small dose of DDC, however, there were no significant changes in the contents of either NA and DA, though the analgesic effect of morphine was potentiated.

Discussion. Contrary to our expectation, on injection of DDC into rats no parallel was found between the initial change in CA content in the whole brain and potentiation of morphine analgesia. Our results, in connection with the findings of TAKAGI et al.[3] and with data of reserpine[5,12] on mice, at least, may indicate that a certain level of DA must be maintained in the brain to manifest the morphine analgesia.

However, there seems to be another possibility that the potentiating action of DDC is connected with impairment of turnover rate of CA in the brain, because, as is well known, DA-β-hydroxylase is inhibited by DDC[6], and because in rat treated with reserpine which suppresses the morphine analgesia, on the contrary, DA-β-

Table I. Effect of DDC on morphine analgesia in rats estimated by the tail clip method

Pretreatment	Hours after DDC	Analgesia[a] (morphine: 10 mg/kg, s.c.)
Saline		74.1 ± 7.7 (26)[b]
DDC, 500 mg/kg × 2, s.c.[c]	4	125.1 ± 9.0 (12)[d]
DDC, 350 mg/kg, i.p.	1	127.0 ± 5.7 (20)[d]

[a] Analgesia was expressed as the difference between the sum of the lag times after morphine and saline administration. [b] Values are means ± standard error. The number of animals used is shown in parenthesis. [c] Animals received 2 injections of DDC 7 and 4 h before the test. [d] Difference from the control statistically significant, $P < 0.01$.

Table II. Effect of DDC on morphine analgesia estimated by the hot plate method

Pretreatment	Hours after DDC	Analgesia[a] (morphine: 10 mg/kg, s.c.)
Saline		262.0 ± 14.5 (8)[b]
DDC, 500 mg/kg × 2, s.c.[c]	4	379.0 ± 24.0 (10)[d]
DDC, 350 mg/kg, i.p.	1	360.3 ± 10.0 (8)[d]

References [a-d] are the same as in Table I.

Table III. Effect of DDC on the catecholamine content of the whole brain of rats

Pretreatment	Hours after DDC	Catecholamine content (μg/g) Noradrenaline	Dopamine
Saline		0.31 ± 0.02 (4)[a]	0.77 ± 0.03 (4)
DDC, 500 mg/kg × 2, s.c.[c]	4	0.12 ± 0.03 (4)[b]	0.89 ± 0.07 (4)
DDC, 350 mg/kg, i.p.	1	0.25 ± 0.03 (5)	0.81 ± 0.05 (5)

[a] Values are means ± standard error. The number of experiments is shown in parenthesis. [b] Difference from the control statistically significant, $P < 0.01$. [c] Animals received 2 injections of DDC 7 and 4 h before the test.

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hydroxylase and dopa decarboxylase activities in the brain were accelerated[13].

On the other hand, some another properties of DDC must be considered, i.e. the inhibition of enzyme activities, including those involved in morphine destruction.

Our results show that DDC potentiates the morphine analgesia and that the initial changes in CA content itself in whole brain by DDC do not seem to be responsible for this phenomenon.

Zusammenfassung. Nach DDC-Vorbehandlung wurde bei männlichen Ratten eine deutliche Potenzierung der Morphinanalgesie beobachtet. Es konnte nachgewiesen werden, dass dieses Phänomen nicht durch die nach DDC-Gabe auftretende Veränderung des CA-Gehaltes im Gehirn bedingt ist.

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Aggression in Mice Associated with Changes in the Monoamine-Metabolism of the Brain

It is known that the administration of L-DOPA and psychotomimetic drugs may provoke an aggressive behaviour in certain animals (Valzelli[1]). However, in principal the biochemical background creating aggressive attitudes is unknown.

Previously Lycke and Roos[2] reported that the *Herpes simplex* virus (HSV) encephalitis in mice will cause raised synthesis of dopamine (DA) as well as 5-hydroxytryptamine (5-HT). These studies suggested the use of specific inhibitors for DA and 5-HT. In one series of experiments the mice were treated with the methylesterhydrochloride of DL-*p*-chlorophenylalanine (H 69/17), an effective inhibitor for the hydroxylation of tryptophane to 5-hydroxy-tryptophane.

Swiss albino mice of our own laboratory breed were inoculated intracerebrally with a mouse-brain-adapted strain (St 2 Gbg 11) of HSV (15 LD_{50}). H 69/17 was given by injection, 400 mg/kg, one day prior to virus inoculation and then daily for 4 days, after which the animals were sacrificed and the brains analyzed for the contents of DA, 5-HT, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA). Determinations of DA and HVA were made spectrophotofluorometrically according to the methods described by Carlsson and Waldeck[3] and Andén et al.[4]. Assays of 5-HT and 5-HIAA were made according to Andén and Magnusson[5] and Roos[6], respectively. Infective virus was titrated by determining the number of plaque-forming units (pfu) on monolayer cultures of GMK cells, overlayed with Eagle's complete medium containing 3% bovine serum and 1% methylcellulose.

The HSV infected animals, treated with H 69/17, demonstrated at 4 days after the virus infection marked excitation and, in addition, an aggressive behaviour. Thus, they not only revealed the characteristic jumpiness and sensitivity to light and sound effects, usually found during the excitatory stage of the disease, but were frequently found involved in fights. No aggressiveness was noted in mice only inoculated with HSV or only treated with H 69/17, although both these groups of animals showed signs of excitation. Table I presents the results of assays of monoamines and infective virus.

The rise in DA synthesis due to the herpetic encephalitis is reflected in the increased concentrations of HVA. The inhibitory effect of H 69/17 on 5-HT synthesis appears from the 5-HT as well as the 5-HIAA concentrations. No inhibitory effect on synthesis of HSV by H 69/17 was observed.

As the aggressiveness was assumed to be conditioned by an increased DA synthesis and a simultaneous reduction of the formation of 5-HT, a series of experiments

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