Table III. Ulcerogenic action of thiohydantoins, thioamides and mercaptohiazoline in rats

Compounds	Dose (mg/kg)	Route	No. of rats	Grades of duodenal ulcer[a] —	+	++	+++
3-Allyl-5-isobutyl-2-thiohydantoin	175	s.c.[c]	5	0	0	1	4 (1)[b]
3-Allyl-5-isobutyl-hydantoin	250	s.c.	5	5	0	0	0
3-Ethyl-5-isobutyl-2-thiohydantoin	250	s.c.	5	0	3	0	2 (1)
3-Ethyl-5-isobutyl-hydantoin	250	s.c.	5	5	0	0	0
Thiobenzamide	300	s.c.	5	0	0	2	3 (1)
Benzamide	1000	s.c.	5	5	0	0	0
α-Ethylthioiso-nicotinamide	300	oral	5	0	1	1	3
Thioacetamide	500	s.c.	5	5	0	0	0
Thiobarbituric acid	500	s.c.	5	5	0	0	0
Thiouracil	500	s.c.	5	5	0	0	0
Mercaptothiazoline	500	s.c.	5	0	0	3	2

[a] See the note of Table I. [b] Numerals in parentheses indicate the number of rats with perforating ulcer. [c] s.c., subcutaneous.

shallow ulcers frequently appeared in the antrum. These pathological features resemble those of the gastroduodenal lesion in rats caused by infusion of secretagogues[3]. Therefore, it is reasonable to assume that hypersecretion of acid from the parietal cells is also responsible for the pathogenesis of thiohydantoin-induced duodenal ulcer This assumption is supported by the fact that vagotomized rats were quite refractory to the ulcerogenic effects of the compound (Table II).

As shown in Table III, duodenal ulcers could be induced in rats not only by thiohydantoins but also by other compounds such as thiobenzamide, α-ethylthioisonicotinamide and mercaptothiazolin, while 3-allyl- or 3-ethyl-5-isobutylhydantoin, benzamide, thiouracil or thiacetamide did not produce any gastroduodenal alterations. This finding may be relevant to the clinical evidence which indicates the occurrence of gastric distress or abdominal discomfort by α-ethylthioisonicotinamide[7,8] or 3-allyl-5-isobutyl-2-thiohydantoin[9].

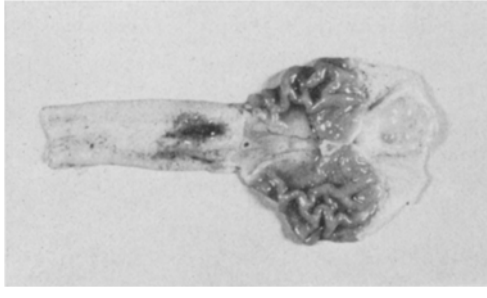

Fig. 1. The stomach and duodenum from a rat 24 h after a s.c. injection of 250 mg/kg of 3-allyl-5-isobutyl-2-thio-hydantoin. An elongated deep ulcer and small shallow ulcers are seen in the duodenum. 3 small ulcers appear in the antrum of the stomach.

Recently Cook et al.[10] and Malen et al.[11] have shown that certain thiocarboxamides such as 2-phenyl-2-(2-pyridyl) thioacetamide and 2-(2-pyridyl) butanothioamide possess a specific gastric antisecretory property in animals. Lee et al.[12] have demonstrated antigastrin activities of 2-phenyl-2-(2-pyridyl)-thioacetamide and allied compounds in animals. In contrast with the present finding, these data suggest the possibility that certain thiocarboxamides possess anti-ulcer effects. It is of interest that these structurally-related compounds can exert different effects on gastroduodenal physiology. At present, the structure-activity relationship remains to be established.

Zusammenfassung. Es wird gezeigt, dass in Ratten mit 3-Allyl-5-isobutyl-2-thiohydantoin und ähnlichen Substanzen Duodenalulzera hervorgerufen werden können und dass die Bildung von Ulzera durch eine vorhergehende Vagotomie verhütet werden kann.

M. Matsuura, Y. Hayashi and H. Katayama

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553 (Japan),
25 May 1973.

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Evidence that Morphine Increases Dopamine Utilization in Corpora Striata of Rats

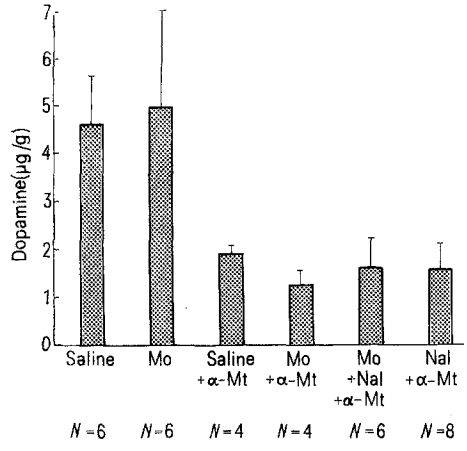
In rats, Kuschinsky and Hornykiewicz[1] observed a dose-dependent increase of striatal homovanillic acid (HVA) concentration after morphine treatment. This effect was explained by an increased dopamine (DA) turnover; other explanations were rather unlikely. However, since no absolutely safe method to estimate brain dopamine turnover exists as yet, I decided to study striatal dopamine turnover by a second, independent approach. Andén et al.[2] used blockade of catecholamine synthesis by α-methyl-*p*-tyrosine, a potent inhibitor of tyrosine hydroxylase[3], as a tool for measuring turnover in the catecholamine neurons. Alter inhibition of dopamine synthesis, the rate of depletion should be proportional to the 'impulse flow' within the neuron: the higher

the activity, the greater the depletion. Therefore, studies of this kind were performed with morphine-treated rats.

Materials and methods. Male, albino Wistar rats (Meyer-Arend, Bad Salzuflen) of 130–180 g were used. The following drugs were applied: morphine hydrochloride, naloxone hydrochloride and DL-α-methyl-*p*-tyrosine (α-MT). Morphine and naloxone were dissolved in saline and injected s.c., α-MT was dissolved in 0.5 *N* HCl, and the pH adjusted to about 2 by adding solid sodium bicarbonate. Part of the substance precipitated, but the suspension was easily injectable i.p. in this form. For doses and injection schedules see the Figure.

For DA estimation, the rats were decapitated and the striata were quickly removed, frozen on dry ice and stored frozen until used. Then the tissues were thawed, homogenized in 4% perchloric acid containing 0.02 *M* EDTA, centrifuged at 4 °C and about 4000 × *g*, and the supernatant was adjusted to pH 5.8 using $KHCO_3$. After another centrifugation at 4000 × *g*, the supernatant was poured onto Dowex columns (50W-X8, 200–400 mesh, H^+, length about 3.5 cm, diameter about 0.5 cm), and eluted with 2.0 *N* HCl, as described by HÄGGENDAL[4]. The dopamine eluate was evaporated, redissolved in 0.02 *N* HCl, and the dopamine was estimated fluorimetrically[5]. The mean ($N = 5$) recovery was 57 ± 10.7% (S.D.). The experimental values are not corrected for recovery.

Results and discussion. Morphine, in a dose of 10 mg/kg s.c., did not alter dopamine levels in the striata (Figure), although this dose induced clear signs of muscular rigidity, hypokinesia and catalepsy; α-MT depleted the striatal dopamine levels by about 60%, and morphine significantly increased this depleting effect. GUNNE et al.[6] obtained similar results with the combination of α-methyl-*p*-tyrosine methylester and morphine. However, they measured the dopamine concentration in the whole brain and not in the striata. Our own result is also in good agreement with the findings of CLOUET and RATNER[7], who observed an increased and accelerated incorporation of intracisternally injected ^{14}C-tyrosine into the dopamine of rat striata.



Dopamine concentration in rat striata. The height of the columns indicates the DA concentration (µg/g striatal tissue ± S.D.). Morphine (Mo) [10 mg/kg s.c.] or saline, when given alone, were injected 60 min before sacrifice of the animals. When given in combination with α-MT (α-MT), morphine, naloxone [1 mg/kg s.c.], the combination of both morphine and naloxone, or saline were injected 15 min before injection of α-MT [200 mg/kg i.p.]. The naloxone injection was repeated 1 and 2 h after the morphine injection. The animals were sacrificed 3 h after α-MT application. Compared with saline + α-MT, the morphine + α-MT-treated group differs significantly [$P < 0.01$, Student's *t*-test].

After application of the morphine antagonist naloxone, morphine was completely ineffective; it did not induce any additional depletion. Naloxone seemed slightly to increase the α-MT-induced depletion of DA, although this effect was not statistically significant. This observation supports the previous assumption[1] that, in the striatal dopaminergic system, naloxone acts as a partial agonist with a relatively low intrinsic activity. Furthermore, the above results confirm the previous conclusion that morphine specifically increases the utilization (and thus the turnover) of DA in the striata.

Because morphine did not change the DA levels, it can be concluded that the DA synthesis was increased, too. Although in rat brains, chlorpromazine and other neuroleptics, similarly to morphine, increase DA turnover in doses which do not affect the DA concentration[8,9], and although both morphine and neuroleptics decrease the dopaminergic neurotransmission, it was concluded earlier[1] that morphine and neuroleptics act by different mechanisms. In neuroleptics, it is almost generally accepted that, after blockade of DA receptors, the dopaminergic neurons, as a compensatory mechanism, activate their DA synthesis and increase the release from the nerve terminals[10], resulting in an increased 'impulse flow' within the neurons.

Morphine, on the other hand, does not seem to block DA receptors, and it is still a matter of question whether the 'impulse flow' – in contrast to the DA utilization – in striatal dopaminergic neurons is in fact increased. The primary site of action of morphine seems to be a presynaptic one and might be some kind of 'diversion' of newly synthesized DA from storage sites to sites of catabolism, leading to a lack of the amine at the receptor sites, and resulting in a central dopamine deficiency syndrome with symptoms such as hypokinesia, catalepsy and muscular rigidity.

Zusammenfassung. Nach Hemmung der Dopaminsynthese beschleunigte Morphin bei Ratten die Abnahme der Dopaminkonzentration im Corpus striatum. Dieser Effekt, der durch Naloxon gehemmt wurde, lässt sich durch eine Erhöhung des Dopamin-Umsatzes im Corpus striatum unter Einfluss von Morphin erklären.

K. KUSCHINSKY[11]

Abteilung Biochemische Pharmakologie, Max-Planck-Institut für experimentelle Medizin, Hermann-Rein-Strasse 3, D–34 Göttingen (W.-Germany), 4 May 1973.

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