Transfer of Induced Tolerance to Morphine and Promazine by Brain Homogenate

Intraperitoneal injections of brain homogenates or brain extracts from morphine-tolerant rats have been reported to decrease the responsiveness of mice to morphine for more than 8 days[1]. As a supplement to an investigation on the mechanisms of induced tolerance to promazine[2], experiments have been performed in order to find out (1) whether the transfer of induced tolerance to morphine is found also when the transfer is made intraspecifically (from tolerant mice to normal mice), and (2) whether induced tolerance to promazine can be transferred by injection of brain homogenates.

3 experimental precautions were considered important: (1) the test and control groups were matched on the basis of their pre-experiment scores, (2) not pooled but individual brain homogenates were used in order to assess their variation range, and (3) the observers did not know to which group each mouse belonged. The animals which were used for the preparation of the brain homogenates are in the following called 'donors', and those which received the homogenate injections, 'recipients'.

Tolerance to morphine was induced by s.c. injections of 15, 50 and 100 mg/kg of morphine hydrochloride/day to male NMRI mice, each dose level being used for 1 week. The 2 groups of control animals received the corresponding injections of saline. After this treatment the responsiveness to morphine of both the tolerant and of those of the first control group was tested by injecting 6 mg/kg of morphine and assessing the analgesic action by Haffner's method[3] 30 and 60 min after the injection, and the hypothermic action by measuring the colonic temperature 1 h after the injection of 10 mg/kg of morphine[2]. The animals of the second control group, which had not received even a single injection of morphine, were used as control donors.

The donor animals were decapitated, their brains removed, immediately frozen on dry ice, and homogenized in a glass homogenizer. The brain homogenate from each animal was injected i.p. into a recipient male NMRI mouse. The groups of recipients had been previously matched on the basis of the hypothermic action of morphine. The third group received an injection of an equivalent amount (1 ml) of saline.

The analgesic and hypothermic actions of morphine in the recipients were tested as previously 1–5 days after the transfer.

Tolerance to promazine was induced similarly, the dose used being 50 mg/kg of promazine hydrochloride daily for 3 weeks. Only the hypothermic effect of promazine was tested. Otherwise, the procedure was similar to that described above.

From morphine-tolerant donors, 12 out of 15 and 3 out of 15 controls were insensitive to the analgesic action of morphine 60 min after the challenge dose. 4 from the recipients of the homogenate from the tolerant animals, 5 from the controls, and 3 from the saline controls were insensitive to the analgesic action of morphine 1 day after transfer. 3 days after the transfer, the corresponding numbers were 6, 5 and 5. Thus there were no significant differences between the recipient groups in the sensitivity to the analgesic action of morphine. Similar results were obtained in another experiment which was performed using the hot plate technique for the assessment of the analgesic action.

The hypothermic effect of morphine (10 mg/kg) was completely eliminated by chronic treatment with this drug. In control animals, the colonic temperature was decreased by 1.4 °C in response to this dose of morphine. The colonic temperatures of the recipients 1 h after the injection of 10 mg/kg of morphine are presented in the Table. The hypothermic effect appears to be similar in all recipient groups both on the first and third day after the transfer. The colonic temperature before the injection was on the average 37.8 °C in all groups.

The hypothermic effect of promazine was reduced in those donors which had received this drug repeatedly. The colonic temperature of the tolerant donors was lowered by 0.9 °C, and that of the control donors by 4.8 °C in 1 h by a dose of 10 mg/kg of promazine. The results presented in the Table show that the hypothermic effects of promazine were similar in all recipient groups. The first of the experiments concerning the transfer of induced promazine tolerance was performed using the Polytron (high frequency) homogenization (1 min), the second without the freezing of the brains after their removal.

In conclusion, the intraspecific transfer of the induced tolerance to morphine and promazine has been proved to be unsuccessful. The consideration of the experimental precautions taken in this study seems to be important in the re-examination of the interspecific tolerance transfer as well as of the learning transfer effects produced by injections of brain extracts or homogenates[4].

The colonic temperature (°C) of the recipients of different homogenates or saline 1 h after the injection of 10 mg/kg of morphine or promazine at various intervals after the transfer injections

Donors	No.	Recipients		No.
Morphine experiment		1 day	3 days	
Control	15	36.6 ± 0.2	36.3 ± 0.3	15
Tolerant	15	36.7 ± 0.1	36.1 ± 0.2	15
Saline		36.8 ± 0.1	37.0 ± 0.1	15
Promazine experiment 1			2 days	
Control	15		33.6 ± 0.3	15
Tolerant	15		33.4 ± 0.2	15
Saline			33.3 ± 0.2	15
Promazine experiment 2		1 day	4 days	
Control	10	33.1 ± 0.2	33.8 ± 0.2	10
Tolerant	10	32.9 ± 0.4	34.0 ± 0.4	10

Zusammenfassung. Gehirnhomogenate zeigten, i.p. in unbehandelte Mäuse eingespritzt, gegenüber analgetischen und hypothermischen Wirkungen von Morphin oder Promazin toleranter Mäuse keine Toleranz-Übertragung. Die Ergebnisse widersprechen den Resultaten früherer Untersuchungen.

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[1] G. UNGAR, Fedn Proc. Fedn Am. Socs exp. Biol. *24*, 548 (1965).
[2] R. TIRRI, Annls Acad. Sci. Fenn. A IV *103*, 1 (1966).
[3] C. BIANCHI and JOLANDA FRANCESCHINI, Brit. J. Pharmac. Chemother. *9*, 280 (1954).
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