Transfer of morphine tolerance in rats by brain extracts

Ungar & Cohen (1966) first described the transfer of tolerance to mice with injections of brain extracts from morphine tolerant animals. Other investigators (Tirri, 1967; Smiths & Takemori, 1968; Cox & Ginsburg, 1969) were unable to transfer tolerance in this way. We now report the transfer of tolerance from rats to rats.

Thirty white male rats, 155–250 g, had morphine hydrochloride morning and night intraperitoneally; the starting dose was 20 mg/kg twice daily. On 3rd, 6th, 9th, 12th, 15th and 18th days, the dose was increased by daily increments of 40 mg/kg so that on the 18th day the daily dose was 280 mg/kg. This dose was maintained until the 23rd day. Ten control rats received equivalent volumes of saline by the same route every morning and night. On the 23rd day two rats from the control group and six rats from the morphine-treated group were given 25 mg/kg of nalorphine hydrochloride intraperitoneally and showed obvious signs of the abstinence syndrome (Kaymakçalan & Woods, 1956).

On day 24, 16 h after the last dose of morphine, 10 rats were decapitated and their brains transferred immediately to 75 ml of cold acetone and homogenized with a Böhler homogenizer for 5 min at 15 000–17 000 rev/min, the homogenizer being cooled externally with ice cold water. The volume of the extract was made to 150 ml with acetone and centrifuged (Sorvall RC2B) for 2 h at 39 000 *g* and −2 to −5°. After 18–24 h at −2° the supernatant was discarded and the precipitate mixed with 50 ml saline and homogenized again at 15 000–17 000 rev/min for 3 min.

Intact rats received intraperitoneally 0·8–1·0 ml of the brain extract, either obtained from the rats rendered tolerant to morphine or from the saline treated group, and their pain reaction time tested with the hot plate (57·5 ± 0·5°) method (Johannesson & Woods, 1964), before and 24 h after injection of the extract. After second testing they received morphine (6 mg/kg i.p.) and 90 min later were tested again.

We observed an obvious tolerance to the analgesic effect of morphine in animals treated with the brain extract from tolerant animals (Table 1), results which are in accordance with those of Ungar & Cohen (1966).

Table 1. *Reaction time to thermal stimuli* (seconds ± s.e.)

Group	First testing: Control (before injection)	Second testing: 24 h after injection of brain extract and 1st testing	Third testing: 90 min after injection of morphine, 6 mg/kg, and 2nd testing
Control	7·33 ± 0·56 n = 6	—	13·1 ± 1·01* n = 6
Normal rats brain extract injected group	8·0 ± 0·18 n = 6	8·0 ± 0·43 n = 6	12·8 ± 1·44† n = 6
Tolerant rats brain extract injected group	7·9 ± 0·45 n = 10	9·2 ± 0·85 n = 10	9·6 ± 0·60‡ n = 10

* $P < 0{\cdot}0005$ when compared with initial value of same group.
‡ $P < 0{\cdot}0025$ when compared with initial value of same group.
† Non-significant when compared with initial value of same group.

Inhibition of protein synthesis can prevent the development of tolerance (Cohen, Keats & others; 1965; Loh, Way & Shen, 1969; Cox & Osman, 1970); it is reasonable to assume the transfer of material of a protein nature. Our experiments shed no light on the precise actions of the transferred material.

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Ultrastructural changes induced by pregnenolone nitrile in the rat liver

In rats, intoxication with indomethacin, digitoxin (Selye, 1970a), cyclophosphamide (Selye, 1970b) and various other drugs can be prevented by pregnenolone nitrile (3β-hydroxy-20-oxo-5-pregnene-16α-carbonitrile). So far, this compound has been found to be the most active inhibitor of intoxication among over 300 steroids tested in our laboratory. Its ability to cause liver hypertrophy and increased pentobarbitone clearance in the blood is consistent with the view that this steroid nitrile acts through microsomal enzyme induction (Selye, 1970a,b). Therefore, it seemed of interest to determine whether this compound causes ultrastructural alterations in the hepatocytes.

Female ARS/Sprague-Dawley rats (Madison, Wisconsin, U.S.A.) of average weight 100 g and maintained on freely available Purina Laboratory Chow and tap water were used. Pregnenolone nitrile (10 mg in 1·0 ml of water) was administered orally, by soft rubber catheter, twice daily for 5 days. The animals were killed by destruction of the medulla oblongata on the 6th day, 16 h after the last gavage. A section of the liver was excised, minced, fixed in Millonig's osmium solution and processed for electron microscopic studies, as described elsewhere (Kovacs, Blascheck & Gardell, 1970; Gardell, Blascheck & Kovacs, 1970).

Pregnenolone nitrile-treated animals exhibited a marked proliferation of the smooth-surfaced, with a relative decrease of the rough-surfaced, endoplasmic reticulum (Fig. 1A and B). It could not be ascertained whether this increase was due to degranulation and transformation of the rough-surfaced into the smooth-surfaced endoplasmic reticulum, or whether it represented *de novo* synthesis. Long, smooth-surfaced lamellae were seen in some places; these were the originally ribosome-studded membranes which had undergone degranulation. The mitochondria were somewhat swollen and, in some cases, they assumed a homogeneous appearance with the disappearance of the cristae. Lipid content increased moderately, and the microvilli in the bile canaliculi seemed to be hypertrophied.

Proliferation of the smooth-surfaced endoplasmic reticulum is not a specific effect of pregnenolone nitrile; it is also induced by various other compounds, such as phenobarbitone (Fouts & Rogers, 1965), tolbutamide (Remmer & Merker, 1965), spironolactone (Kovacs & others, 1970), norbolethone (Gardell & others, 1970) and certain other steroids (Horvath, Kovacs & others, 1970). It has been assumed that this change indicates activation of various microsomal drug-metabolizing enzymes in the liver (Fouts & Rogers, 1965; Conney, 1967). However, further investigations are required to clarify the pathophysiological significance of smooth-surfaced reticulum hypertrophy and of the role played by pregnenolone nitrile in causing proliferation of smooth membranes.