the depressant actions of amines on cerebral cortical neurons involve a calcium-dependent mechanism.

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REFERENCES

ARMSTRONG, C. M. & BINSTOCK, L. (1964). *J. gen. Physiol.*, **48**, 265–277.

HURWITZ, L., BATTLE, F. & WEISS, G. B. (1962). *Ibid.*, **46**, 315–332.

ISHIDO, T. (1962). *Jap. J. Leg. Med.*, **16**, 214–232.

KALANT, H. (1970). In: *Alcohols and Derivatives*, p. 189–236 Editor: (Tremoliers, J.) Oxford, Pergamon Press.

KRNJEVIĆ, K. (1971). In: *Methods in Neurochemistry*, p. 129–172 Editor: (Fried, R.) New York, Dekker, M.

PHILLIS, J. W., LAKE, N. & YARBROUGH, G. G. (1973). *Brain Res.*, **53**, 465–469.

SEEMAN, P., CHAU, M., GOLDBERG, M., SAUKS, T. & SAX, L. (1971). *Biochim. biophys. Acta*, **225**, 185–193.

Restoration of morphine analgesia in morphine-tolerant rats after the intraventricular administration of 6-hydroxydopamine

A definitive relation between morphine tolerance and the central adrenergic neurons has not been established. Chronic administration of morphine may affect the intraneuronal catecholamine storage mechanisms, since repeated administration of morphine interferes with the depletion of brain noradrenaline and dopamine levels by reserpine (Gunne, Jonsson & Fuxe, 1969) and also with the decrease in brain dopamine levels after administration of 6-hydroxydopamine (6-OHDA) into the lateral ventricles (Nakamura, Kuntzman, & others, 1972). In morphine-tolerant animals, the ability of morphine to decrease brainstem noradrenaline levels was lost (Maynert, 1968). These results suggest that there may be a correlation between morphine analgesia and release of brain noradrenaline. In addition, morphine-induced depletion of noradrenaline has been observed only in the hypothalamus (Vogt, 1954; Moore, McCarthy & Borison, 1965) mesencephalon, diencephalon and spinal region (Reis, Rifkin & Corvelli, 1969), indicating differences in regional sensitivity. Further, the electrolytic destruction of the dorsomedial (DMH) or ventromedial hypothalamic nuclei (VMH) is known to abolish an established tolerance to morphine (Kerr & Pozuelo, 1971a, b). These areas are rich in nerve endings of the ventral noradrenaline neuron pathway (Ungerstedt, 1971). The effects of morphine on the central adrenergic neurons may also be related to behavioural changes elicited by morphine such as motor excitation and catalepsy as well as to the analgesic action itself.

We have examined the effect on morphine analgesia in morphine tolerant rats of administration of 6-OHDA into lateral ventricle or into the VMH or DMH areas of the brain.

For measuring analgesic activity, the tail-flick method described by Nakamura, Kuntzman & others (1973) was used.

To obtain rats tolerant to morphine, male Wistar rats (Royal Hart strain), 250 g, were injected intraperitoneally 4 times daily (7 and 11 a.m. and 3 and 7 p.m.) with

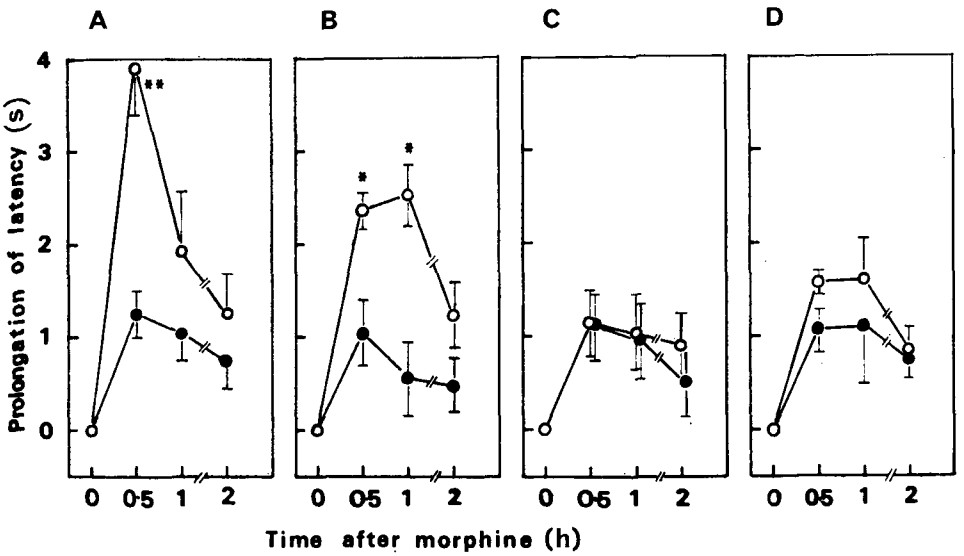

FIG. 1. Effects of the bilateral administration of 6-OHDA into the lateral ventricles, dorsomedial hypothalamic or ventromedial hypothalamic nuclei in morphine-tolerant rats, on morphine analgesia. Morphine-tolerant rats (8 per group) (10 mg kg^{-1}, 4 times daily for 4 days) were treated with 6-OHDA into both of the lateral ventricles (total = 200 g in 20 μl) or into both of DMH or VMH (total = 20 g in 2 μl). Each control received a vehicle solution into the corresponding sites in the brain. Morphine analgesia was measured by the tail-flick response to 5 mg kg^{-1} of morphine on day 5. ● morphine tolerant controls; ○ in A are naive rats to morphine, and in B–D are morphine-tolerant animals after the 6-OHDA treatment. The ordinate is prolongated latency period of the tail-flick response to heat from preinjection time in each group. 8 animals per group.

* Significant difference ($P < 0{\cdot}025$) from morphine tolerant controls; ** $P < 0{\cdot}001$.

morphine hydrochloride (10 mg kg^{-1}; calculated as free base) for 4 days. Morphine analgesia was determined after an intraperitoneal injection of 5 mg kg^{-1} (instead of 10 mg kg^{-1}) at 7 a.m. on day 5. 90% of the animals showed tolerance to morphine analgesia; these were divided into 4 groups. Two h later, they were anaesthetized with ether and 100 μg of 6-OHDA HBr (total: 200 μg), dissolved in 10 μl of ice cold Merlis solution (Merlis, 1940) containing 10 μg ascorbic acid, was bilaterally administered into the lateral ventricles by means of a stereotaxically guided needle to destroy the adrenergic neurons. Bilateral doses of 6-OHDA administered into the DMH or VMH were 10 μg (total = 20 μg) dissolved in 1 μl of Merlis solution containing 1μg of ascorbic acid. After the 6-OHDA, the scheduled doses of morphine were administered. On day 6, one day after the 6-OHDA administration, morphine analgesia (5 mg kg^{-1}, i.p. at 7 a.m.) was again measured. Four h later the animals were exsanguinated and brain noradrenaline and dopamine were adsorbed on activated alumina (Anton & Sayre, 1962) and determined fluorimetrically (Shellenberger & Gordon, 1971). Each group contained 8 animals.

There was no difference in the latency period (mean $\pm$ s.e.) of the tail-flick response to heat when naive controls (4·9 $\pm$ 0·25) and morphine-tolerant animals (4·9 $\pm$ 0·3 s) were compared. But, analgesia after giving 5 mg kg^{-1} of morphine to the chronically treated animals was markedly decreased (Fig. 1A). One day after 6-OHDA the analgesic response to morphine (5 mg kg^{-1}) of the morphine-tolerant rats was almost completely restored (Fig. 1B). In contrast, 6-OHDA placed in the DMH or VMH caused no significant effect on the analgesia in morphine-tolerant rats (Fig. 1C, D).

The administration of 6-OHDA into the lateral ventricles of chronically morphin-

Table 1. *Effects of the administration of 6-hydroxydopamine into the brain on brain catecholamine contents in acutely and chronically morphinized rats in groups of 8–10 animals.* Chronically morphinized rats (10 mg kg^{-1} 4 times daily for 4 days) were treated by 6-OHDA into both of the lateral ventricles (total = 200 μg in 20 μl) or both of the DMH or VMH by 6-OHDA (total = 20 μg in 2 μl). The brain catecholamines of a pair of groups were measured 24 h after 6-OHDA and 4 h after morphine (5 mg kg^{-1} i.p.).

Site of 6-OHDA administration		Noradrenaline in ng g^{-1} Hypothalamus	Medulla-pons	Residual brain[a]	Dopamine in ng g^{-1} Residual brain[a]
Untreated Controls	I[b]	1477 ± 127	529 ± 40	281 ± 24	515 ± 36
	II[b]	1303 ± 178	479 ± 39	264 ± 26	498 ± 29
Lateral ventricles	I	303 ± 58	255 ± 37	83 ± 12	464 ± 25
	II	106 ± 22**	135 ± 42*	92 ± 9	440 ± 55
DMH	I	156 ± 31	237 ± 31	171 ± 15	436 ± 89
	II	111 ± 27	190 ± 29	147 ± 11	640 ± 73
VMH	I	516 ± 127	401 ± 58	213 ± 25	479 ± 29
	II	614 ± 59	314 ± 59	171 ± 38	503 ± 51

[a] Residual parts of the brain minus the cerebellum.
[b] I means a single administration of morphine; II, chronic treatments of morphine.
* Significant difference ($P < 0.05$) from the corresponding acutely morphinized controls; ** $P < 0.005$.

ized rats decreased the noradrenaline content in the hypothalamus and medulla pons much more than it did in acutely morphinized rats (Table 1). In contrast, there was no difference in the depleted noradrenaline contents in the brain regions after the administration of 6-OHDA into DMH or VMH of controls and chronically morphinized rats. The dopamine content in the residual brain, containing the neostriatum, did not change one day after 6-OHDA administration, as has been described previously (Uretsky & Iversen, 1970: Nakamura & others, 1972). These results suggest that the restoration of morphine analgesia in tolerant rats is related more closely to the depletion of noradrenaline from the brainstem area than to that of brain dopamine.

Since 6-OHDA-induced destruction of the central adrenergic neurons causes denervation supersensitivity (Strada, Uzunov & Weiss, 1971; Uretsky & Schoenfeld, 1971; Ungerstedt, 1971; Nakamura & Thoenen, 1972), it is probable that the recovery of morphine analgesia in morphine-tolerant animals after 6-OHDA may be at least partly induced by postsynaptic supersensitivity to noradrenaline released by morphine in the brainstem. A preliminary observation we made indicated that a scheduled morning dose of morphine (80 mg kg^{-1}, i.p.) in chronically morphinized rats elicited the motor excitation or convulsion when the animals were intraventricularly treated by 6-OHDA (200 μg) on the previous night. The morphine-tolerant rats were prepared by the cumulative administration of morphine twice daily, morning and evening, from the starting dose of 20 mg kg^{-1} day^{-1} until 160 mg kg^{-1} day^{-1}. The results suggest that the release of brain noradrenaline is responsible for the motor excitation or convulsion following morphine treatment in tolerant animals as well as for the morphine analgesia. The administration of 80 mg kg^{-1} i.p. to naive rats with or without 6-OHDA treatment into the lateral ventricle killed the animals by depression of respiration within 1 h.

It is less probable that there is participation of 5-HT neurons with the 6-OHDA-induced restoration of morphine analgesia in tolerant animals, since it is known that the administration of 6-OHDA into the brain has no significant effect on brain

contents of 5-HT (Bloom, Algeri & others, 1969; Breese & Traylor, 1971; Uretsky & Iversen, 1970).

In conclusion, the intraventricular administration of 6-OHDA restored the analgesic action of morphine in morphine-tolerant rats, suggesting that the development of tolerance to morphine analgesia may be at least partly due to changes in the central noradrenaline neurons.

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REFERENCES

ANTON, A. H. SAYRE, D. F. (1962). *J. Pharmac. exp. Ther.*, **138**, 360–375.
BREEZE, G. R. & TRAYLOR, T. D. (1971). *Br. J. Pharmac.*, **42**, 88–99.
BLOOM, F. E., ALGERI, S., GROPPETTI, A., REVUELTA, A. & COSTA, E. (1969). *Science*, **166**, 1284–1286.
GUNNE, L.-M., JONSSON, J. & FUXE, K. (1969). *Eur. J. Pharmac.*, **5**, 338–342.
KERR, F. W. L. & POZUELO, J. (1971a). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **30**, 375.
KERR, F. W. L. & POZUELO, J. (1971b). International Symposium on drug tolerance, addiction, abuse and methadone treatment. New Orleans, 40 abs.
MAYNERT (1968). *The addictive states*, pp. 89–95. Winkler, A. Baltimore: Williams & Wilkins.
MERLIS, J. K. (1940). *Am. J. Physiol.*, **131**, 67–72.
MOORE, K. E., MCCARTHY, L. E. & BORISON, H. L. (1965). *J. Pharmac. exp. Ther.*, **148**, 165–175.
NAKAMURA, K., KUNTZMAN, R., MAGGIO, A. & CONNEY, A. (1972). *J. Pharm. Pharmac.*, **24**, 484–487.
NAKAMURA, K., KUNTZMAN, R., MAGGIO, A., AUGULIS, V., LEWINSON, T. M. & CONNEY, A. H. (1973). *Psychopharmac.*, in the press.
NAKAMURA, K. & THOENEN, H. (1972). *Ibid.*, **24**, 359–372.
REIS, D. J., RIFKIN, M. & CORVELLI, A. (1969). *Eur. J. Pharmac.*, **9**, 149–152.
SHELLENBERGER, M. K. & GORDON, J. H. (1971). *Analyt. Biochem.*, **39**, 356–372.
STRADA, S. J., UZUNOV, P. & WEISS, B. (1971).*Pharmacologist*, **13**, 257.
UNGERSTEDT, U. (1971). *6-Hydroxydopamine and catecholamine neurons*, pp. 101–127. Editors: Malmfors, T. & Thoenen, H. North-Holland.
URETSKY, N. J. & IVERSEN, L. L. (1970). *J. Neurochem.*, **17**, 269–278.
URETSKY, N. & SCHOENFELD, R. (1971). *Nature*, **234**, 157–159.
VOGT, M. (1954). *J. Physiol.*, **123**, 451–581.

Dopamine-induced relaxation of isolated arterial strips

The lack of a suitable isolated organ preparation has hampered characterization of the proposed dopamine vascular receptor (van Rossum, 1965; Goldberg, 1972). We undertook the present investigation to search for an isolated arterial strip that would relax upon application of dopamine. We concentrated our efforts on dog renal and mesenteric arteries, since vasodilatation has been observed in these vascular beds in this species (McNay, McDonald & Goldberg, 1963; Eble, 1964; McNay & Goldberg, 1966).

The method used has been previously described (Toda, Usui & others, 1972). Isolated vessels (0·5 to 2 mm outside diameter) were cut into spiral strips of approximately 25 mm in length and were then fixed vertically in a muscle bath of 20 ml capacity containing nutrient solution (in mM concentrations: Na^+, 162·1; K^+, 5·4; Ca^{2+}, 2·2; Cl^-, 157·0; HCO_3^-, 14·9; dextrose, 5·6). The bathing medium was maintained at