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Prevention of the reserpine effect on rat salivary gland noradrenaline by inhibitors of monoamine oxidase and catechol-*O*-methyl transferase

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The roles of monoamine oxidase and catechol-*O*-methyl transferase for the inactivation of noradrenaline after reserpine treatment have been investigated in rat salivary glands *in vivo*. Inhibition of monoamine oxidase by nialamide retarded the reserpine-induced disappearance of noradrenaline in the glands, whereas inhibition of catechol-*O*-methyl transferase by 4-tropolone acetamide did not. However, when both enzymes were inhibited, reserpine produced a significantly slower disappearance rate of noradrenaline compared with inhibition of monoamine oxidase alone. Thus, after reserpine, most of the noradrenaline is metabolized by monoamine oxidase. When this enzyme is inhibited catabolism by catechol-*O*-methyl transferase appears to play a part in the reduction of noradrenaline induced by reserpine.

Reserpine is known to deplete the tissue stores of catecholamines (Carlsson, Rosengren & others, 1957; Burn & Rand, 1958), and this is thought to be due to it blocking the uptake of the amines into storage granules. Reserpine has also been reported to alter catecholamine metabolism. Thus, there is an increased catabolism of catecholamines by monoamine oxidase (MAO) after reserpine treatment (for review see Carlsson, 1965). Evidence has been presented that in rat salivary glands this increased deamination occurs intraneuronally (Jonason, 1969a). In the present experiments the relative roles of MAO and catechol-*O*-methyl transferase (COMT) for the inactivation of noradrenaline in rat salivary glands after reserpine have been investigated. This has been done by measuring the reserpine-induced disappearance of noradrenaline after inhibition of MAO or COMT, or both.

EXPERIMENTAL

Adult male Sprague-Dawley rats, about 200 g, were treated with reserpine, 1 mg/kg, intraperitoneally. Some of the animals were pretreated with the MAO inhibitor nialamide (100 mg/kg, i.p.) 2 h before, or the COMT inhibitor 4-tropolone acetamide (100 mg/kg, i.p.) 30 min before the reserpine administration. Another group of animals received a combination of these two enzyme inhibitors as pretreatment. The rats were killed by a blow on the head at different times after the reserpine administration. The submaxillary plus the sublingual glands on each side were removed as soon as possible, weighed and homogenized in 10 ml 0·4N perchloric acid by an "Ultra-Turrax" homogenizer. The noradrenaline was determined spectrophotofluorometrically after cation exchange chromatography (Bertler, Carlsson & Rosengren, 1958).

Table 1. *Effect of inhibition of monoamine oxidase or catechol-O-methyl transferase, or both, on the reserpine-induced noradrenaline disappearance rate in rat salivary glands.* Rats were treated with reserpine (1 mg/kg, i.p.) and the noradrenaline content of the submaxillary plus the sublingual glands was determined at different time intervals after the injection. Some of the animals were pretreated with the monoamine oxidase inhibitor nialamide (100 mg/kg, i.p.) 2 h before the reserpine administration or the catechol-*O*-methyl transferase inhibitor 4-tropolone acetamide (100 mg/kg, i.p.) 30 min before the reserpine administration. Another group of the animals received a combination of the two drugs as pretreatment.

s.e. represents the standard error of the mean and n represents the number of experiments.

		Noradrenaline content μg/g tissue after reserpine treatment for:			
		0 h	4 h	6 h	8 h
Reserpine	Mean	1·183	0·014	0·007	0·006
	s.e.	0·068	0·0071	0·0042	0·0043
	n	8	5	4	10
Nialamide + reserpine	Mean	—	0·235	0·122	0·108
	s.e.	—	0·021	0·0114	0·034
	n	—	5	6	10
4-Tropolone acetamide+reserpine	Mean	—	0·015	—	0·001
	s.e.	—	0·0057	—	0·00047
	n	—	6	—	10
Nialamide + 4-tropolone acetamide + reserpine	Mean	—	0·383	0·236	0·103
	s.e.	—	0·045	0·045	0·034
	n	—	6	6	10

RESULTS

The results are presented in Table 1. Four h after the reserpine administration the noradrenaline content of the salivary glands was reduced to a very low concentration which was maintained for the rest of the investigated time intervals. Inhibition of COMT by 4-tropolone acetamide did not affect this reserpine-induced noradrenaline reduction. Pretreatment of the rats with nialamide (100 mg/kg, i.p.) does not significantly increase the noradrenaline content in the salivary glands within 2 h (Jonason, 1969a). However, after inhibition of MAO by nialamide, reserpine produced a much slower disappearance rate of the salivary gland noradrenaline, resulting in significantly higher noradrenaline content in the MAO-inhibited glands than after reserpine alone at all time intervals investigated ($P < 0{\cdot}001$).

Pretreatment of the animals with both the MAO inhibitor nialamide and the COMT inhibitor 4-tropolone acetamide resulted in a further reduction of the reserpine-induced noradrenaline disappearance rate. The noradrenaline content of the salivary glands was found to be significantly higher in the nialamide plus 4-tropolone acetamide-treated glands than in the nialamide-treated glands 4 and 6 h after reserpine ($P < 0{\cdot}005$ and $P < 0{\cdot}025$, respectively). Eight h after reserpine treatment there was no difference between the two pretreatments.

DISCUSSION

Kalsner & Nickerson (1969) recently reported that interference with intraneuronal storage by reserpine results in active amine being inactivated by COMT rather than by MAO in rabbit aortic strips. However, these results were obtained after adding noradrenaline exogenously to muscle baths. From the above experiments, dealing with endogenous noradrenaline levels after reserpine, it is obvious that MAO inhibition significantly reduced the rate of noradrenaline decrease after reserpine whereas inhibition of COMT did not affect the reserpine-induced noradrenaline reduction. The prevention of the reserpine effect on the noradrenaline by a MAO inhibitor but not by a COMT inhibitor supports the view that after reserpine treatment most of the noradrenaline is metabolized by means of MAO (Carlsson & others, 1957; Carlsson & Hillarp, 1962; Kopin, 1964; Malmfors, 1965). This oxidative deamination after reserpine treatment has been shown to occur intraneuronally (Jonason, 1969a).

However, the role of COMT has also been partially revealed in the present investigation. If both MAO and COMT were inhibited, the prevention of the reserpine effect on noradrenaline was augmented. Thus, after MAO inhibition followed by reserpine treatment, the noradrenaline is to a certain extent 3-*O*-methylated by COMT. This enzyme has been shown to be localized to the parenchymal cells in rat salivary glands (Jonason, 1969b,c). Jonason (1969a) has demonstrated that after treatment with nialamide the reserpine-induced noradrenaline reduction is slower in atrophied rat salivary glands compared with intact ones. Since there is a severe loss of COMT activity in atrophied glands (Jonason, 1969b,c), the reason for this difference may well be the lack of COMT. The data from the present investigation support this interpretation. Thus, it seems probable that after MAO inhibition followed by reserpine treatment the noradrenaline leaves the adrenergic nerves and is 3-*O*-methylated by means of parenchymal COMT or is transported unchanged from the neuroeffector units by the blood.

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REFERENCES

BERTLER, Å., CARLSSON, A. & ROSENGREN, E. (1958). *Acta physiol. scand.*, **44**, 273–292.

BURN, J. H. & RAND, M. J. (1958). *J. Physiol., Lond.*, **144**, 314–336.

CARLSSON, A. (1965). *Handbuch der exp. Pharmak.*, Bd XIX, 529–592. Ed.: V. Erspamer. Berlin-Heidelberg-New York: Springer Verlag.

CARLSSON, A. & HILLARP, N.-Å. (1962). *Acta physiol. scand.*, **55**, 95–100.

CARLSSON, A., ROSENGREN, E., BERTLER, Å. & NILSSON, J. (1957). *Psychotropic Drugs*, 363–372. Editors: Garattini, S. and Ghetti, V. Amsterdam: Elsevier.

JONASON, J. (1969a). *Acta physiol. scand.*, **75**, 73–77.

JONASON, J. (1969b). *Ibid.*, **76**, 299–311.

JONASON, J. (1969c). *Acta pharmac. tox.*, **27**, 60–72.

KALSNER, S. & NICKERSON, M. (1969). *Br. J. Pharmac.*, **35**, 394–405.

KOPIN, I. J. (1964). *Pharmac. Rev.*, **16**, 179–191.

MALMFORS, T. (1965). *Acta physiol. scand,*. **64**, *Suppl.* 248, 1–93.