Beta-Adrenergic Blocking Agents as Potent Antagonists of Mescaline-Induced Contractions in the Rat Uterus

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Summary. In the isolated rat uterus, mescaline induces contractions that are notably antagonized by catecholamines, by β-adrenergic stimulants and certain β-adrenergic blocking agents as well as by chlorpromazine, amitriptyline and methysergide.

Some years ago, in the course of routine examination of the effects of different compounds on isolated organs, it was noted that various phenylalkylamine hallucinogens[1] induce rapid contractions of smooth-muscle organs, e.g. the small intestine of the guinea-pig and the rat uterus. Mescaline, for instance, elicited reproducible contractions of the rat uterus at concentrations ranging from 0.3 to 30 μg/ml.

In order to characterize the mode of action of mescaline on this organ, a series of pharmacological agents were tested with regard to their capacity to antagonize contractions evoked by this hallucinogen. In the course of these investigations, it was found that certain β-adrenergic blocking agents, but also β-adrenergic stimulants, were capable of inhibiting mescaline-induced contractions in the rat uterus at very low concentrations. Of the psychopharmacologically active drugs examined, chlorpromazine proved by far the most active antagonist of mescaline. An account of this work is given below.

Materials and methods. Uterine horns from virgin rats were mounted as described previously[2] in an organ bath containing de Jalon's solution at 28 °C which was aerated with a mixture of CO_2 (5%) and oxygen (95%). Contractions were induced at intervals of 10 min by adding mescaline sulphate at a final concentration of 10 μg/ml. After a 30 sec exposure, the mescaline was washed out. The drugs to be tested for antagonistic activity were added to the bath fluid 2 min before mescaline. In parallel experiments on separate uterine preparations, the capacity of the compounds to antagonize contractions elicited by serotonin creatinine sulphate (final concentration 0.1 μg/ml) was also determined. The drugs used are listed in the Table.

Results. The results are summarized in the Table. The following drugs counteracted the contractions elicited by mescaline in the rat uterus at very low to fairly low concentrations: a) adrenaline and noradrenaline; b) β-adrenergic stimulants (e.g. isoprenaline, terbutaline); c) certain β-adrenergic blocking agents (e.g. oxprenolol, the D-isomer being the active moiety of the racemate, pronethalol); d) some psychopharmacological agents (e.g. chlorpromazine, amitriptyline, benzoctamine); e) a serotonin antagonist (methysergide).

The specific parasympatholytic compounds atropine, scopolamine and oxyphenonium bromide, and also the potent spasmolytic agents papaverine and adiphenine, were either only active at comparatively high concentrations or even inactive in the highest dose tested. Likewise, the sympatholytic drugs (phentolamine, hydergine, dibenamine) were only feebly active. Of the various psychoactive drugs examined in addition to chlorpromazine, only amitryptiline exerted antagonistic activity at a comparatively low concentration, whereas notably the three antidepressant drugs (imipramine, desipramine, maprotiline) were only active at high concentrations. The antihistamine tripelennamine was found to be inactive in this respect, as was also the local anaesthetic drug dibucaine. When the capacity of the drugs examined to antagonize mescaline-induced contractions is compared with their respective antagonistic effects against serotonin-induced contractions, it is evident that these two activities do not generally run parallel. The contrast is particularly marked among the compounds displaying pronounced antagonistic activity against mescaline-induced contractions of the rat uterus. Thus, adrenaline is a distinctly more potent antagonist of serotonin than of mescaline; on the other hand, chlorpromazine is some 300 times less active against serotonin than against mescaline, whereas isoprenaline, oxprenolol and methysergide are equally active against both types of contraction. It is conceivable that the contraction of the

Drug	Inhibition of contractions elicited by Mescaline (IC_{50} ± SE)[a]	Serotonin (IC_{50})[b]
Adrenaline hydrochloride	0.01 ± 0.003	0.0003
Noradrenaline hydrochloride	0.01 ± 0.008	0.2
Isoprenaline hydrochloride	0.0001 ± 0.0002	0.0001
Salbutamol	0.0001 ± 0.0003	n.d.
Terbutaline sulphate	0.0012 ± 0.0003	n.d.
Phentolamine hydrochloride	6 ± 0.7	20
Hydergine methanesulfonate	1	1
Dibenamine hydrochloride	0.3 ± 0.02	0.02
Oxprenolol hydrochloride	0.006 ± 0.002	0.003
D-isomer	0.005 ± 0.003	n.d.
L-isomer	0.1 ± 0.06	n.d.
Propranolol hydrochloride	3 ± 1.8	10
Pronethalol hydrochloride	0.06 ± 0.004	> 1
Metoprolol hydrochloride	10[c]	n.d.
Oxyphenonium bromide	10–30	30
Scopolamine hydrobromide	100[c]	0.08
Adiphenine hydrochloride	3	2
Papaverine hydrochloride	5	3
Tripelennamine hydrochloride	30	10
Benzoctamine hydrochloride	0.3 ± 0.007	2
Maprotilin hydrochloride	1 ± 0.08	0.2
Imipramine hydrochloride	2 ± 0.05	1
Desipramine hydrochloride	6	3
Amitriptyline hydrochloride	0.03 ± 0.007	0.3
Dibenzepin hydrochloride	10	5
Chlorpromazine hydrochloride	0.0003 ± 0.0002	0.1
Methysergide hydrogen maleate	0.01 ± 0.003	10
Chlordiazepoxide hydrochloride	60	0.3
Dibucaine hydrochloride	10	20

The concentrations listed refer to the salt, the nature of which is indicated in the first column.
[a] The IC_{50} was determined graphically on semi-logarithmic paper on the basis of a 3 to 4 point assay, and the SE calculated according to Lord when $n \geqq 3$. All other data represent the results of 1–2 determinations. [b] n = 1–2. [c] Inactive at the concentration shown. n.d. = not done.

[1] P. BRAWLEY and J. C. DUFFIELD, Pharmac. Rev. *24*, 31 (1972).
[2] R. JAQUES and B. SCHÄR, Helv. physiol. Acta *15*, 134 (1957).

rat uterus caused by mescaline is due to the release of endogenous mediators, such as serotonin or acetylcholine, that stimulate smooth muscle. Histamine can be ruled out a priori, since it is well known that the rat organs are highly resistant to this amine; this is supported by the lack of effect of tripelennamine. The fact that those drugs causing the most potent inhibition of mescaline-induced contractions were only rarely as active against contractions elicited by serotonin, would seem to eliminate this amine as a potential mediator of the effect of mescaline. Acetylcholine likewise cannot be involved in view of the inactivity of parasympatholytic drugs.

It is difficult to interpret the findings presented above in terms of the possible therapeutic mechanisms of action of the drugs examined, and it is somewhat surprizing that mescaline causes contractions of a smooth-muscle organ such as the uterus. We also have no straightforward explanation for the fact that our findings are somewhat at variance with those of COSTA[3], who did not observe a direct effect of mescaline on the same organ, but only a potentiation of serotonin-induced contractions. On the other hand, it is perhaps relevant that some of the psychoactive drugs examined, e.g. chlorpromazine and amitryptiline, are potent inhibitors of mescaline at peripheral receptors, especially if one assumes that a similar antagonism might take place at mescaline receptors in the central nervous system. Moreover, the unexpected finding that some β-adrenergic blocking agents, notably oxprenolol, antagonize the action of mescaline, could have some bearing on the clinical observations that such drugs in high dosages exert beneficial effects in mania and agitated psychoses[4] or display general psychotropic activities[5]. The capacity of sympathomimetic substances to antagonize mescaline seems to be best explained as being one facet of their non-specific spasmolytic action. In this regard, it may suffice to mention the fact that adrenaline is a highly potent antagonist of contractions induced by trypsin in the rat uterus[2].

[3] E. COSTA, Proc. Soc. exp. Biol. Med. *91*, 39 (1956).
[4] W. GRÜTER, Symposium Betablocker und Zentralnervensystem, St. Moritz 1976, in press.
[5] D. J. GREENBLATT and R. I. SHADER, Curr. ther. Res. *14*, 615 (1972).

Effect of Acetylcholine on Melanophores of *Rana tigrina*

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Summary. Acetylcholine produced melanin aggregation and blanching of skin colour in *Rana tigrina*, the common Indian frog. The effects were more prolonged in frogs pretreated with an anticholinesterase agent. Acetylcholine effects were not antagonized by either *m*-cholinolytic (atropine) or *n*-cholinolytic (pentolinium) agents, but were markedly inhibited by procaine. The results have been discussed in the light of the well-known membrane-stabilizing effect of procaine.

No phenomenon of nature has probably attracted more attention or has been investigated from more diverse angles than that of colour changes in animals. In amphibians the integumentary colour changes are produced by variations in the skin melanophores, which form a very delicate and responsive system, constantly undergoing changes in response to alterations in the external environment and internal homeostasis[1]. The skin colour of *Rana tigrina*, the common Indian frog, varies from a dirty dark brown to a light yellowish-green colour. These variations in skin colour result from intracellular movement of melanin granules within the melanophores. Dispersion of melanin granules from a perinuclear position out into the melanophore processes, results in darkening of skin colour, while aggregation of melanin granules from the melanophore processes to a perinuclear position causes lightening of skin colour[2].

No reports are available on the effect of acetylcholine, the cholinergic transmitter, on melanophores of *Rana tigrina*. The present investigation reports the effect and possible mode of action of acetylcholine on skin colour and melanophores in this species.

Material and method. Studies were conducted on adult frogs, of either sex, weighing between 150 and 300 g. Frogs were anaesthetized with pentobarbitone sodium (50 mg/kg in ventral lymph sac). Drugs, dissolved in 0.6% saline in a fixed volume, were administered through cannulated left branch of thoracic aorta. The animals were kept submerged throughout the experiment in 0.6% saline for adequate cutaneous respiration. The dorsal skin colour was observed by naked eye and the most lateral web of the left hind limb was observed under low power ($\times 60$) microscope. Individual melanophores were measured with a Leitz micrometer eyepiece by noting the maximum vertical and horizontal diameters. 5 such melanophores were measured and the mean 'melanophore size index' was recorded as the:

$$\frac{\text{maximum vertical} \times \text{horizontal diameter (in } \mu\text{m)}}{100}.$$

Since the cell outline of the melanophores was only distinct when they were in the contracted state, care was taken to measure only the span of the pigment and not the cell boundary. In effect, therefore, the variable recorded is the linear disposition of the pigment in melanophore processes. The melanophore size index was noted before and at different time periods after drug administration and the difference in pre and post drug peak effect was taken into consideration for statistical analysis by Student's *t*-test.

Results and discussion. Results are summarized in the Table. Acetylcholine (0.1 mg/kg) produced centripetal movement of melanin granules, leading to aggregation and resulting in blanching of skin colour. The effect was transient and passed off in 5–10 min. However, in frogs

[1] S. J. HOLMES, *The Biology of the Frog*, 4th edn. (Macmillan Co., New York 1934), p. 191.
[2] J. D. TAYLOR and M. E. HADLEY, Z. Zellforsch. mikrosk. Anat. *164*, 282 (1970).