feeding intervals. Bound gossypol concentration in the lungs increased accumulatively through the 28-day feeding interval but demonstrated a decline at the end of the 35-day feeding interval.

Free gossypol levels in the kidney, spleen and lungs were greatest during the shorter feeding periods and generally displayed decreased levels at the end of the 28- and 35-day feeding periods. With the exception of the 14-day feeding period, the free gossypol concentration in the liver remained nearly constant.

Although this study was of relatively short duration, it clearly indicates the wide distribution of gossypol in the organs of the rat. It also adequately establishes the relationship between the duration of gossypol ingestion and the accumulation of gossypol in the organs of the rat. If gossypol is an accumulative poison, its concentration in each organ should vary with the duration of the ingestion period. This was found to be the case for bound and total (bound plus free) gossypol concentrations in the liver and spleen, but not for the kidneys and lungs. It is apparent that total gossypol levels in the kidneys and lungs reach a maximum and then decrease as the length of the feeding period is increased. A possible explanation may be that there is a shift of accumulated gossypol in the kidneys and lungs to other organs. The presence of two reactive carbonyl groups on the gossypol molecule and the multiplicity of chemicals available in each organ for interaction with gossypol allows for a variety of bound forms of gossypol to exist. It is possible that these compounds vary widely in their motility within the organism and account for an extremely complex accumulation pattern.

Interaction of Atropine or Methylatropinium with Four Effects of Two Cholinergic Drugs

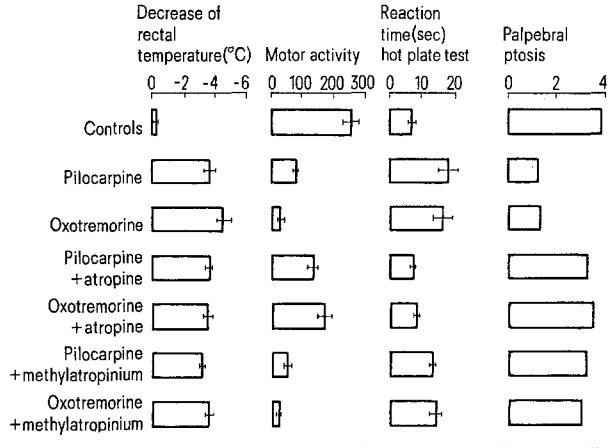
P. Simon, R. Chermat and J. R. Boissier

Département de Pharmacologie, Faculté de Médecine Pitié-Salpétrière, 91, Boulevard de l'Hôpital, F-75634 Paris-Cedex 13 (France), 22 September 1975.

Summary. In mice, pilocarpine – or oxotremorine – induced decrease in locomotor activity and increase of the reaction time to pain were antagonized by atropine and not by methylatropinium. Identical doses of atropine and methylatropinium suppressed the antagonism of the cholinergics towards reserpine-induced palpebral ptosis. Cholinergics-induced hypothermia was not clearly antagonized by atropine or methylatropinium.

The sedative effects of the cholinergic drugs are antagonized by atropine but not by its quaternary derivative. So is the decrease in locomotor activity[1], the catalepsy[2], the suppression of an avoidance-conditioning[3], the potentiation of the barbiturates effects[4] and the antagonism of the amphetamine-induced hyperactivity[5].

However, Spencer[6] and Janssen and Niemegeers[7] have shown that higher doses of the quaternary derivatives of atropine-like drugs could exert the same antagonism as atropine towards the effects of the cholinergic drugs.



Influence of atropine or methylatropinium on four effects of pilocarpine or of oxotremorine. The doses of pilocarpine and of oxotremorine were different according to the test (see materials and methods). The total length of the horizontal bars represents 2 SEM. Locomotor activity results are expressed by the mean of beams crossed by one mouse during 30 min. Palpebral ptosis was appreciated from 0 to 4 according to Rubin et al.[12].

Among the various common effects in mice of two cholinergic drugs, oxotremorine and pilocarpine[8], we chose four of them; in order to dissociate the central component from the peripheral component of these effects we tested whether atropine or methylatropinium could antagonize the effects of oxotremorine and pilocarpine in these four experimental situations at doses of the anticholinergic agent which do not modify these tests when injected alone[9].

Materials and methods. For all experiments, groups of 10 male mice (19–23 g) were used (six for the palpebral ptosis). Atropine or methylatropinium (1 mg · kg^{-1}) were always given i.p. 15 min before pilocarpine or oxotremorine. Rectal temperature was measured 30 min after pilocarpine (64 mg · kg^{-1}) or oxotremorine (0.25 mg · kg^{-1}) administration. Locomotor activity was measured between the 30th and the 60th min after pilocarpine (4 mg, kg^{-1}) or oxotremorine (0.06 mg · kg^{-1}) administration. During this period, each mouse was placed in an actograph box[10]. The reaction time to a nociceptive stimulus

[1] L. S. Harris, Biochem. Pharmac. *8*, 92 (1961).
[2] B. Costall and J. E. Olley, Neuropharmacology *10*, 297 (1971).
[3] C. C. Pfeiffer and E. H. Jenney, Ann. N.Y. Acad. Sci. *66*, 753 (1957).
[4] A. Tsujimoto, T. Dohi, M. Ikeda and T. Nishikawa, Archs int. Pharmacodyn. Thér. *212*, 264 (1974).
[5] C. D. Proctor, J. L. Potts, L. G. Ashley and B. A. Denefield, Archs int. Pharmacodyn. Thér. *167*, 61 (1967).
[6] P. S. J. Spencer, Br. J. Pharmac. Chemother. *25*, 442 (1965).
[7] P. A. J. Janssen and C. J. E. Niemegeers, Psychopharmacologia *11*, 231 (1967).
[8] R. Chermat, P. Simon and J.-R. Boissier, J. Pharmac., Paris *5*, suppl. 2, 18 (1974).
[9] J. Malatray and P. Simon, Thérapie *27*, 153 (1972).
[10] J.-R. Boissier et P. Simon, Archs int. Pharmacodyn. Thér. *158*, 212 (1965).

was appreciated using the 'hot plate test'[11]. This test was performed 30 min after pilocarpine (16 mg · kg^{-1}) or oxotremorine (0.125 mg · kg^{-1}) administration.

Palpebral ptosis was induced by i.p. administration of reserpine (2.5 mg · kg^{-1}) 4 h before the administration of the cholinergics (pilocarpine, 16 mg · kg^{-1} or oxotremorine, 0.25 mg · kg^{-1}). The ptosis was appreciated[12] 30 min after the cholinergic drug.

Results and discussion. The results are indicated on the Figure. The cholinergic-induced hypothermia was not antagonized either by atropine (1 mg · kg^{-1}) or by methylatropinium (1 mg · kg^{-1}). Pilocarpine-induced hypothermia was not antagonized by higher doses (4 mg · kg^{-1} i.p.) of atropine ($-2.8\,°C \pm 0.3$) or of methylatropinium ($-3.4\,°C \pm 0.2$). Oxotremorine-induced hypothermia was antagonized significantly more by atropine ($-2.0\,°C \pm 0.4$) than by methylatropinium ($-4.2\,°C \pm 0.3$).

The decrease in activity induced by the two cholinergic drugs was clearly antagonized by atropine but was not modified by methylatropinium. The increase in the reaction time to the nociceptive stimulus induced by pilocarpine or oxotremorine was suppressed by atropine, whereas it was not significantly modified by methylatropinium. Atropine or methylatropinium inhibited the antagonism of the two cholinergic drugs towards the palpebral ptosis induced by reserpine. The two effects which seem related to the sedative action of cholinergics (decrease in locomotor activity, increase of the reaction time to pain) were antagonized by atropine and not by methylatropinium, and the results are in keeping with those of other authors (vide supra). Identical doses of atropine and methylatropinium suppressed the antagonism of oxotremorine and pilocarpine towards reserpine-induced palpebral ptosis. Therefore this antagonism seems to be at a peripheral level.

On the other hand, the oxotremorine- and pilocarpine-induced hypothermia was not modified by the same doses of atropine or methylatropinium which can be active in the other tests. However, the doses of oxotremorine and pilocarpine were higher than the doses necessary to obtain the three other effects. It was possible partly to antagonize the oxotremorine-induced hypothermia but not the pilocarpine-induced hypothermia, with 4 times higher doses of atropine (4 mg · kg^{-1} i.p.) but not with methylatropinium (4 mg · kg^{-1} i.p.). Thus the hypothermia induced by pilocarpine or oxotremorine does not seem to be due completely to a cholinergic mechanism.

However, the possibility of pharmacological or metabolic interactions between quaternary derivatives of atropine-like drugs and cholinergics must be kept in mind. For instance, KARLEN, TRÄSKMAN and SJÖQVIST[13] showed that the peripheral effects of oxotremorine induced marked changes in the blood flow to different tissues. The antagonism of this peripheral effect by atropine methylnitrate results in lower brain concentration of oxotremorine compared to controls because of a greater effective peripheral volume of distribution.

[11] G. WOOLF and A. D. MACDONALD, J. Pharmac. exp. Ther. *80*, 300 (1944).
[12] B. RUBIN, M. H. MALONE, M. H. VAUGH and J. C. SURKE, J. Pharmac. exp. Ther. *120*, 125 (1957).
[13] B. KARLEN, L. TRÄKSMAN and F. SJÖQVIST, J. Pharm. Pharmac. *23*, 758 (1971).

Melatonin Antagonizes Colchicine-Induced Mitotic Arrest[1]

T. J. FITZGERALD and ANN VEAL

The Florida Agricultural and Mechanical University, School of Pharmacy, Tallahassee (Florida 32307, USA),
9 September 1975.

Summary. Melatonin, in concentrations up to 10^{-3} *M*, showed no effect on mitosis in cultures of HeLa or KB cells. However, when melatonin at 10^{-4} *M* was preincubated with HeLa cells prior to addition of 10^{-7} *M* colchicine, a reduction in the mitotic index, in comparison to colchicine alone, was observed.

It has been recently reported that the pineal hormone, melatonin, exhibits colchicine-like antimitotic activity in *Allium cepa* root hair cells[2]. We have attempted to determine whether this activity would also be displayed in mammalian cell lines.

HeLa cells and KB cells were purchased from Flow Laboratories and grown in Eagle's medium with 10% fetal calf serum. Logarithmically growing cells were exposed to the drug in monolayer for 7 h at 37 °C. At the end of the incubation period the cells were trypsinized off the culture dish, fixed (acetic acid:methanol/1:3), treated with Geimsa stain, and examined for mitotic fugures (500 cells per slide; 2 slides from separate cultures per experiment).

Colchicine produced maximum accumulation of metaphases (22–25%) at 5×10^{-7} *M* in both cell lines. Melatonin, however, even at concentrations of 10^{-3} *M* did not alter the mitotic index from control values (control cultures of both cell lines had a mitotic index of $4 \pm 1.5\%$). It was felt that perhaps melatonin might affect some other aspect of the mitotic cycle. It has been shown, for instance, that cytochalasin B inhibits cytokinesis (mitosis just prior to cell cleavage) through interaction with microfilaments[3]. Therefore, differential counts on the various phases of mitosis were performed, including telophase 1 (complete, bipolar separation of chromosomes) and telophase 2 (pinching of the cytoplasm and reconstruction of the nucleus), for colchicine,melatonin and control cultures. The values obtained are displayed in Table I and show that melatonin has no effect at this concentration (10^{-4} *M*) on any phase of the mitotic cycle in these cells.

In order to assess the effect of melatonin on the antimitotic action of colchicine both agents (colchicine,

[1] This work was supported by PHS Grant No. CA 16425.
[2] S. BANERJEE and L. MARGULIS, Expl Cell Res. *78*, 314 (1973).
[3] H. A. S. VON DEN BRENK and M. G. STONE, Nature, Lond. *251*, 327 (1974).