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EFFECTS OF TRIPERIDOL ON TRANSMISSION AND ON RELEASE OF ACETYLCHOLINE IN THE RAT SYMPATHETIC GANGLION *IN VITRO**

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Abstract—Triperidol (TP) rapidly depresses the postganglionic response evoked by preganglionic stimulation of the superior cervical ganglion (s.c.g.) isolated *in vitro*, under sustained activity. Examination of total acetylcholine (ACh) by bioassay in the s.c.g. and in the incubation medium showed a TP-induced increase of ganglionic ACh coupled with a decrease in the medium. Parallel effects were observed on the labelled-ACh content of ganglia incubated with ^{3}H-choline. These effects are all consistent with the idea that a primary action of TP is to inhibit the release of ACh.

TRIPERIDOL (1-3-(4-fluorobenzoyl)propyl-4-(3-trifluoromethylphenyl)-piperidil-4-ol) (TP) belongs to the family of basic butyrophenones and displays a strong neuroleptic activity.[1–3]

Studies by Janssen,[4] and Buchel and Levy[5] indicated that TP exerts a mainly anti-adrenergic action, both central and peripheral, along with a minor para-sympatholytic activity observed on the isolated rat duodenum.[5] Michalek *et al.*[6] have studied the effects of TP on the intermediate metabolism of carbohydrates and amino acids in rat brain cortex slices incubated *in vitro*. A further study by Michalek *et al.*[7] has shown that 0·2 mM TP decreases by about 80 per cent the amount of acetylcholine (ACh) released from cortex slices incubated in a KCl-rich medium; a significant effect is seen at concentrations as low as 0·002 mM. On the other hand, TP has shown no inhibitory effect on the activity of the enzyme choline–acetyl-transferase extracted from rat brain tissue and assayed *in vitro*.

We have investigated the action of TP on the function of rat superior cervical ganglion incubated *in vitro*, and on the ganglionic content of: (a) labelled ACh formed during incubation with ^{3}H-choline under sustained activity; (b) total ACh, determined by bioassay. By the same assay procedure the ACh released into the medium was determined. These experiments have provided indications about the possible interference of TP with the processes of synthesis, storage and release of ACh.

MATERIALS AND METHODS

Superior cervical ganglia from male Sprague–Dawley rats (180–220 g) were dissected, mounted, incubated and stimulated as described by Larrabee *et al.*[8] The incubation medium was 1 ml of M.E.M.-Eagle (Wellcome Research Laboratories,

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Beckenham, England). The amplitude of the postganglionic action potentials, evoked by preganglionic supramaximal stimulation, was recorded. The observed depression was expressed as a per cent difference between the amplitude of the action potentials recorded before and after TP addition. Before use, TP was dissolved in an excess of lactic acid and neutralized. The same amount of lactate was added to the medium of the control ganglia. This final lactate concentration (44 μg/ml) is lower than its physiological level in rat plasma (90 μg/ml)[9] and has proved to be devoid of any detectable effect on the ganglionic response.

Synthesis and analysis of labelled ACh. In the first series of experiments each ganglion was incubated for 90 min, under repetitive stimulation (10/sec), in a medium adjusted to contain carrier choline (Ch) (1·5 μg/ml)[10] plus radioactive Ch (10 μCi/ml) and TP (0·02 mM final concn). At the end of incubation the ganglion was chilled and rinsed in ice-cold medium, then ACh ("total ACh")[10] was extracted with HCl pH 4 at 100° and separated into two dimensions by thin layer electrophoresis (modification of Potter and Murphy's procedure[11]) followed by chromatography as described by Diamond and Kennedy.[12] Radioactivity of ACh was counted by scintillation spectrometry. A full report of this procedure has been given in a previous paper.[13]

In a second series of experiments, after 2 hr of incubation of ganglia with ^{3}H-choline under repetitive stimulation, TP was added and the incubation was continued for 2 hr under the same conditions.

Bioassay of ACh. Each experiment was carried out simultaneously on four ganglia individually incubated in 0·5 ml of medium and stimulated for 90 min. Two ganglia (experimental) were treated with TP, while two others (controlateral ganglia from the same rats) were the controls. The assay of ACh was carried out, in triplicate, with the extracts from two pooled ganglia on the guinea-pig ileum, according to a procedure reported previously.[14] In a second series of experiments directed to the bioassay of ACh released in the incubation medium, the medium included neostigmine ($1{\cdot}5 \times 10^{-2}$ mM) and at the end of incubation it was acidified with HCl to pH 4.

Products. ^{3}H-choline (Choline chloride(methyl-T), (spec. act. 15·4 Ci/mM)) Radiochemical Centre, Amersham. Trifluperidol R2498 (A1202), Janssen Research Laboratories, Beerse, Belgium and Lusofarmaco, Milano, Italy. Neostigmine methyl sulphate, Roche, Basel.

RESULTS

Action of TP on postganglionic action potentials evoked by preganglionic supramaximal stimulation. When TP (0·02 mM final concn) was added to the incubation medium, the amplitude of the postganglionic action potentials was reduced to 50 per cent within 8 $\pm$ 2 min (mean $\pm$ S.E.M. of eleven experiments); complete suppression of the response was achieved within 1 hr. A typical experiment is illustrated by the graph in Fig. 1.

The 0·02 mM concentration was chosen because of its well known effect on the biochemical processes in brain cortex slices.[7] Lower and higher concentrations (0·01 and 0·04 mM) have shown slower and faster effects, respectively.

Action of TP on the ganglionic content of labelled ACh. As reported in Table 1, the content of ^{3}H–ACh decreased by 33 per cent when TP was added at the beginning of the 90 min-incubation with ^{3}H–Ch, while it increased by 42 per cent in 120 min when TP was added after the previous 120 min incubation with ^{3}H–Ch.

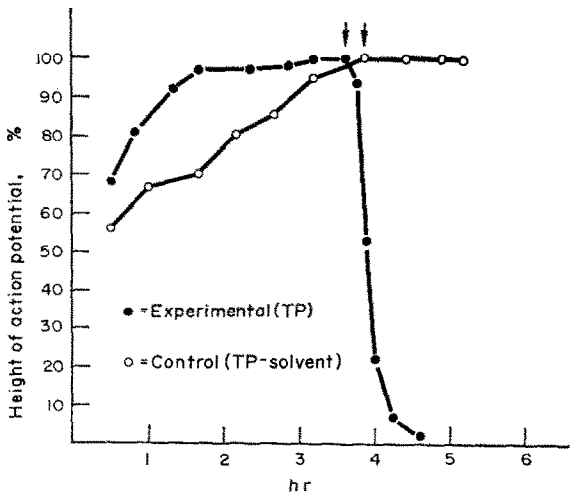

FIG. 1. Effect of 0·02 mM triperidol (TP) on postganglionic action potentials of superior cervical ganglion, evoked by repetitive supramaximal stimulation of the preganglionic nerve (10 stim/sec). % Refers to the height of action potential at the time of TP addition. TP-solvent was added to the control ganglion (open circles).

TABLE 1. LABELLED ACh IN SUPERIOR CERVICAL GANGLION (ISOLATED *in vitro*) INCUBATED WITH AND WITHOUT 0·02 mM TRIPERIDOL

	(n)	Control (dis/min/ganglion)	Experimental (dis/min/ganglion)	Δ% Referred to control ganglion	P (%)
A	(7)	39,464 ± 6920	26,954 ± 5258	−33	>99
B	(5)	52,861 ± 6238	74,891 ± 7855	+42	>99

(A) Both ^{3}H-choline and triperidol were added at beginning of incubation, which lasted 90 min under repetitive stimulation.

(B) After incubation for 2 hr with ^{3}H-choline under repetitive stimulation, triperidol was added and the same conditions were maintained for 2 hr.

(n) Number of experiments.

The values shown are the mean ±S.E.M. Statistical significance of paired differences is calculated on the basis of Student's *t*-test.

TABLE 2. BIOASSAY OF ACh IN SYMPATHETIC GANGLIA AND IN INCUBATION MEDIA WITH AND WITHOUT 0·02 mM TRIPERIDOL UNDER REPETITIVE STIMULATION (10/sec) FOR 90 MIN

	(n)	Control (ng of ACh*/ganglion)	Experimental (ng of ACh*/ganglion)	Δ% Referred to control ganglion	P (%)
Ganglion	(5)	25·4 ± 0·4	38·5 ± 4·9	+49·8 ± 10	>99
Medium	(5)	91·8 ± 4	18·2 ± 2	−80·0 ± 2	>99

(n) Number of experiments.

* Acetylcholine, expressed as acetylcholine chloride.

The values shown are the mean ±S.E.M. Statistical significance of paired differences is calculated on the basis of Student's *t*-test.

Action of TP on the amount of ACh determined by bioassay in the ganglion extract and in the medium. Table 2 shows that TP treatment for 90 min increased the ganglionic content by about 50 per cent, whereas it decreased the amount of ACh released into the medium by about 80 per cent.

DISCUSSION

TP has been shown to cause a prompt and extensive depression of the ganglionic response as well as marked changes in the amounts of ACh found in the ganglion and in the medium. The functional impairment might obviously be ascribed to the action of TP at any level. An effect at the sub- or post-synaptic level might be suggested by the atropine-like action of TP described by Buchel and Levy.[5] This action, however, could be only a minor component of the effect observed by us, because according to Volle,[15] the atropine-sensitive component of the ganglionic synapses is not essential for transmission, even if Watson[16] has demonstrated that atropine, to a certain extent, antagonizes the depolarizing effect of ACh on the ganglionic cells caused by stimulation of the muscarinic receptors. However, since it is known that the function of the presynaptic endings is particularly sensitive to a variety of ionic, metabolic and pharmacological factors, it might be supposed that TP is acting presynaptically. This hypothesis is strengthened by the observed effects of TP on the labelled ACh content of the ganglion and on the amounts of ACh found in the ganglion and in the medium. To explain these effects of TP, reference should be made to the picture outlined by Collier and McIntosh[17] of the synthesis, storage and release of ACh in the superior cervical ganglion of the cat during sustained activity in the presence of labelled choline. In these conditions the ganglionic pool of ACh is labelled at high speed (over 80 per cent in 1 hr) by newly synthesized ACh which replaces the ACh released. Such a replacement is much slower at rest (20–25 per cent in 1 hr). This model allows a common explanation of both the functional and the biochemical effects of TP shown in our experiments, namely that TP causes an impairment of ACh release from the preganglionic nerve endings with consequent loss of synaptic transmission.

In fact, an impairment of the ACh release by TP could well explain the three effects induced by TP when added at the start of incubation with stimulation for 90 min, viz. an increased content of ACh in the ganglion, paralleled with a decrease of the amount of ACh released into the medium and of the ganglionic level of labelled-ACh. In other words, it is suggested that TP, by depressing the release of ACh, has induced its accumulation in the ganglionic pools and reduced its replacement by newly synthesized (labelled) ACh. This view has further support from the experiments (B, Table 1) where TP was added after 2 hr of labelling with ^{3}H–Ch (i.e. when most of the ganglionic ACh was steadily labelled[10,17]). Then a marked increase in the labelled-ACh level (relative to control) is observed, which may correspond to the accumulation (due to an impaired release) of the previously synthesized, heavily labelled ACh. As a whole, no suggestion of a possible direct effect of TP on the synthesis of ACh is provided by the present study.

The observed action of TP on cholinergic nerve endings is therefore to be distinguished from that of hemicholinium, which decreases the amount of released ACh by preventing synthesis; TP must act directly at some stage in the release process.

The present results and their proposed interpretation are in agreement with the finding of Michalek *et al.*,[7] that TP reduces the release of ACh from rat brain cortex slices into the incubation medium. Our results are of interest in view of the recent report by Consolo *et al.*[18] that the level of total ACh is increased in the brain tissue of mice treated with Haloperidol: this compound is closely related to TP (the only difference being a Cl group on the phenyl nucleus instead of CF_3) and is pharmacologically very similar.[19]

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