ACTA PHYSIOLOGICA POLONICA
Vol. XVII, No. 4, 1966

ANNA SZADOWSKA

EFFECT OF DIETHYLAMINOETHYL ESTER OF ACETYLSALICYLIC ACID, OF DIETHYLAMINOETHYL ESTER OF 3-METHYLCOUMARYLIC ACID AND OF SOME OTHER LOCAL ANESTHETICS ON THE ISOLATED SMOOTH MUSCLES OF GUINEA PIG

Chair and Department of Pharmacology, Medical Academy, Łódź
Head: Professor *M. Mazur*, M. D.

SZADOWSKA A.: *Effects of Diethylaminoethyl Ester of Acetylsalicylic Acid of Diethylaminoethyl Ester of 3-Methylcoumarylic Acid and of Some Other Local Anesthetics on the Isolated Smooth Muscles of Guinea Pig.* Acta Physiol. Polon. 17 (4) : 685—693, 1966. — The effect of the hydrochlorides of the diethylaminoethyl ester of acetylsalicylic acid (EDA), diethylaminoethyl ester of 3-methylcoumarylic acid (EDC), novocaine, xylocaine, larocaine, pantocaine and percaine on the smooth muscle of the small intestine, taenia coli and uterus of guinea pigs was studied. EDC, larocaine, pantocaine and percaine exhibited spasmolytic action on smooth muscles of the isolated small intestine and taenia coli of guinea pigs. Novocaine and xylocaine caused relaxation of the small intestine and contraction of the taenia coli. In contrast to the remaining compounds, EDA in low concentrations caused a transient intestinal contraction. It abolished the contractile effect of barium chloride and elicited contractions of the taenia coli of the guinea pig. All the preparations caused contractions of the isolated virgin guinea pig uterus, and only high concentrations caused uterus relaxation.

A variety of esters of diethyl aminoalcohols with aromatic acids, the group of agents many local anesthetics belong to, exert spasmolytic activity towards the smooth muscles [3, 7, 8].

In course of pharmacological studies on newly synthetised diethyl aminoethyl esters of acetylsalicylic acid and of 3-methyl coumarylic acid respectively [5, 7, 8] we have observed that these substances caused dilatation of the smooth muscles of the isolated intestine, bronchi and vessels and contraction of the muscle layer of the uterus of guinea pig, rat and that of rabbit.

Clegg has pointed out that cocaine, butacaine and procaine respectively increased sponaneous contraction of the isolated uterus of guinea pig and as well the susceptibility of the organ towards the action of vasopressin and oxytocin.

In the present communication we determined the effect of a series of local anesthetics — esters of dialkyl aminoalcohols with organic acids and some other chemicals — upon the smooth muscles of the isolated small intestine, taenia coli and the uterus of guinea pig respectively.

The aim of the paper was to establish a relationship if any between the mechanism of action of our newly synthetised compounds and the substances studied by *Clegg* upon the isolated uterus.

METHODS

Studies were performed by the method of *Magnus* on isolated small intestine, taenia coli and uterus of the virgin guinea pig.

Hydrochlorides of following compounds were used: diethyl aminoethyl ester of acetylsalicylic acid (EDA, Edan), diethyl aminoethyl ester of 3-methylcoumoarylic acid (EDC), novocaine, xylocaine, larocaine, pantocaine and percaine respectively. The compounds were applied as dilutions between 10^{-8} to 10^{-3}.

The spasmolytic activity of the compounds was studied employing isolated ileum of guinea pig. The contraction of the intestine was affected by the 10^{-4} solution of barium chloride. The solution of barium chloride was dropped into Ringer's solution every 5 minutes, 4 to 5 times. Thus the reactivity of the intestine towards barium chloride was determined. Thereafter, the anesthetic agent was dropped in followed after two minutes by the addition of barium chloride, this time omitting the washing procedure. Before the next addition of the studied compound, the reaction of the intestine towards barium chloride was again determined. The concentration of the studied preparation was set up so as to decrease the contraction of the intestine by about 50 percent. The precise 50 percent inhibition concentration was calculated by interpolation from 5 independent experiments.

Atropine, DHE and papaverin at 10^{-5} dilution respectively and delizyd and eserine at 10^{-7} dilution respectively were applied to the smooth muscles in order to study the mechanism of action of local anesthetics.

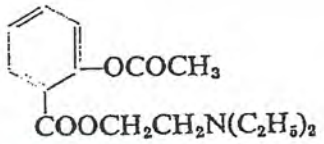
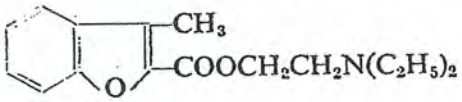
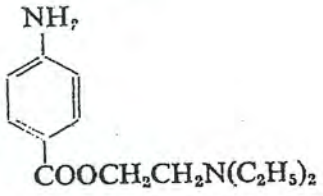
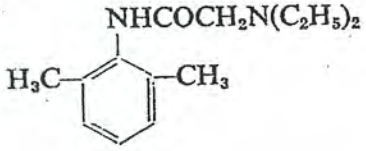
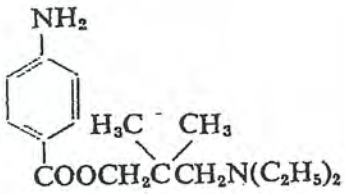
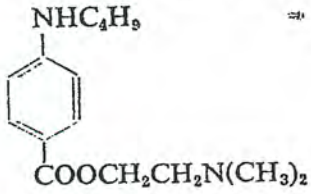
Studies employing organs of reserpine treated guinea pigs were also performed. One group of animals was injected with 0·5 mg/kg of reserpine intraperitoneally every day for 8 consecutive days. Another group of animals was injected with reserpine twice: first time on the day before the experiment (2 mg/kg) and the second time on two hours before killing of animal (4 mg/kg).

RESULTS

Effect on the smooth muscle of the intestine of guinea pig

The local anesthetics used in experiments displayed spasmolytic action upon the smooth muscle of the isolated intestine of guinea pig. EDA was the only exception; at lower concentration (10^{-6}—10^{-4}) the compound caused contraction of the intestine and at high concentration displayed a spasmolytic effect. (Table 1).

Table 1. Determination of the spasmolytic action of local anesthetics on the isolated ileum of the guinea pig

Anesthetic	Formula	Mean concentration of the local anesthetic eliminating in 50 percent the intestinal contraction evoked by the administration of $BaCl_2$
EDA		1 : 112 000
EDC		1 : 1700 000
Novocaine		1 : 182 000
Xylocaine		1 : 17 600
Larocaine		1 : 69 250
Pantocaine		1 : 108 000

Effect upon taenia coli of guinea pig

Novocaine (concentration from 10^{-6} to 10^{-3}) caused contraction of taenia coli. EDA (concentration from 10^{-6} to 10^{-4}) and xylocaine (concentration from 10^{-5} to 10^{-4}) displayed similar effect. At higher concentration both EDA and xylocaine exerted biphasic effect, a transient contraction of taenia coli was followed by its dilatation. EDC, larocaine, pantocaine and percaine caused dilation of taenia coli at concentration higher than 10^{-6} to 10^{-5}.

Papaverin abolished contracting activity of novocaine, EDA and of xylocaine. Atropine, DHE and delizyd were without any effect.

The reactivity of the isolated intestines towards local anesthetics of reserpine treated guinea pigs and of these which were not treated with reserpine was alike.

Effect on the isolated uterus of guinea pig

Novocaine and EDA (concentration 10^{-5} to 10^{-3}) caused contraction of the isolated uterus of guinea pig. Other compounds displayed contracting properties only at low concentration; at high concentration a dilatating effect or a biphasic action was observed.

Among the compounds of possible antagonistic properties only papaverin abolished the contracting activity of the local anesthetics; atropine, DHE and delizyd did not affect the reactivity of the uterus towards these compounds.

Eserine when incorporated into the nourishing solution of the uterus significantly increased the reactivity of the organ towards the local anesthetics.

When the uterus of guinea pigs which had been treated previously with reserpine was used in experiments the organ responded in the same way towards the local anesthetics as the uterus of untreated guinea pigs.

Table 2. Effect of local anesthetics on the isolated smooth muscles of the small intestine, taenia coli and the uterus of guinea pig

Concentration	Novocaine			EDA			Xylocaine			EDC			Larocaine			Panto-caine			Percaine		
	intestine	taenia	uterus	intestine	taenia	uterus	intestine	taenia	uterus	intestine	taenia	uterus	intestine	taenia	uterus	intestine	taenia	uterus	intestine	taenia	uterus
10^{-8}	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
10^{-7}	○	○	○	○	○	○	○	○	○	⊖	○	⊕	○	○	○	○	○	○	⊖	○	○
10^{-6}	⊖	⊕	○	○	⊕	⊕	⊖	○	○	—	○	+	○	○	⊕	—	⊖	⊕	—	○	⊕
10^{-5}	—	+	+	+	+	+	—	⊕	+	—	—	+	—	⊖	+	—	—	+	—	—	+
10^{-4}	—	+	+	+	+	+	—	+	+	—	—	±	—	—	—	—	—	—	—	—	—
10^{-3}	—	+	+	±	±	+	—	±	±	—	—	±	—	—	—	—	—	—			

○ — No action. ⊕ — Weak contraction.
\+ — Contraction. ⊖ — Weak spasmolysis.
— — Spasmolysis. ± — Contraction, spasmolysis.

The results on the reactivity of the smooth muscles of various organs of guinea pigs towards local anesthetics are presented in Table 2 and Fig. 1, 2, 3.

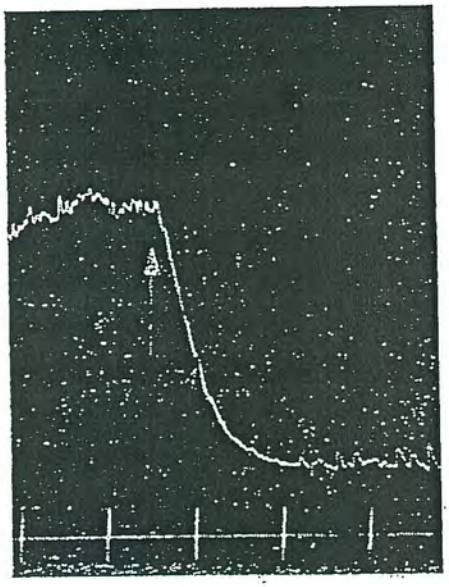


Fig. 1.

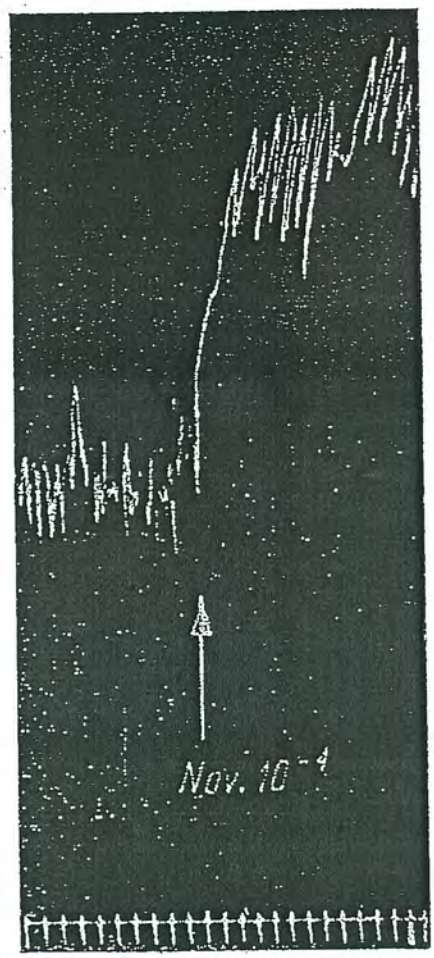


Fig. 2.

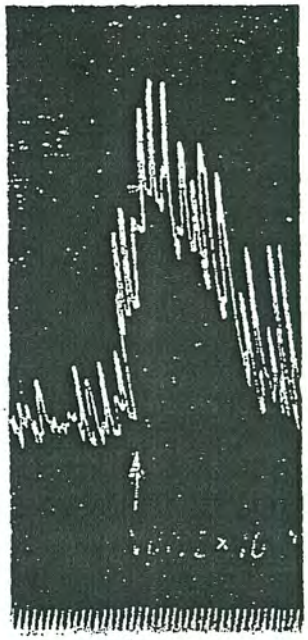


Fig. 3.

Fig. 1. The effect of novocaine on the isolated small intestine of the guinea pig. Time marker 30 sec.

Fig. 2. The effect of novocaine on the isolated taenia coli of the guinea pig. Time marker 30 sec.

Fig. 3. The effect of novocaine on the isolated uterus of the guinea pig. Time marker 30 sec.

DISCUSSION

The results indicate that EDC, larocaine, pantocaine and percaine respectively caused spasmolysis of the smooth muscles of the isolated small intestine and of taenia coli of guinea pigs; novocaine and xylocaine respectively caused dilatation of the small intestine but the contraction of taenia coli. On the other hand, EDA caused only small and transient contraction of the intestine at low concentration but at the same time inhibited the action of barium chloride. Taenia coli was con-

tracted as a result of the application of EDA. All the compounds employed caused contraction of the uterus of the virgin guinea pig; higher concentrations of the same compounds caused on the other hand the dilatation of the organ. Papaverin abolished the contraction of taenia coli and the uterus respectively as effected by local anesthetics.

Clegg has pointed out that the compounds which block the adrenergic system (bretylium, TM 10, guanetidine) increased the susceptibility of the uterus of guinea pig towards contracting stimuli. Similar action was displayed by cocaine, butacaine and procaine. Catechol amines (adrenaline, noradrenaline) caused dilatation of the uterus of guinea pig. The three previously mentioned local anesthetic agents as well as the compounds which block the adrenergic system have not evoked the described effect employing guinea pigs pretreated with reserpine. Therefore *Clegg* has reasoned that the compounds acted *via* blocking the release of catechol amines from the different organs.

In our experience reserpine did not affect the reactivity of the organs of guinea pigs towards the local anesthetic agents. Therefore, the contraction of taenia coli and of the uterus respectively as effected by local anesthetic agents might possess a different mechanism.

Hazard et al. have pointed out that the activity of novocaine persists for a long time although being an ester the compound is rapidly hydrolyzed within the body. The authors have compared the effect of the hydrolysis products of novocaine to that of the intact substance upon the smooth muscles of the bronchi and of the intestine. They have established that para-amino benzoic acid induced spasmolysis of the smooth muscles while diethyl aminoethanol increased the effect of acetylcholine, nicotine and of histamine respectively. Esterification of diethyl aminoethanol with para-aminobenzoic acid masked the contracting effect of diethyl aminoethanol and increased the spasmolytic effect of para-aminobenzoic acid residue.

The spastic effect of diethyl aminoethanol upon the smooth muscles has been also reported by *Kraatz et al.* and also by us [7].

With reference to these findings it occurred to us that the variable reactivity of the smooth muscles towards the tested compounds could have been a result of the presence in the muscles of an unspecific esterase. However since eserine did not affect the spastic effects of the studied esters of diethyl aminoethanol (novocaine, EDA, EDC) upon the uterus of guinea pig it should be concluded that the pharmacological activity of the esters resulted from intact compounds rather than from hydrolysis products.

Bülbring has studied the reactivity of the smooth muscles towards adrenaline and has established that the compound affected cellular mem-

brane directly by bringing about the depolarisation of the membrane. Besides, adrenaline being the activator of phosphorylase, affected also the metabolism of the muscles. The latter effect of adrenaline speeds up the breakdown of glycogen and liberates some energy. The energy may be utilised either for the contraction of the muscles or for the active transport of ions across the cellular membrane. This in turn, leads to the stabilisation of the membrane, lowering of its susceptibility and to hyperpolarisation.

According to the hypothesis the effect of adrenaline upon the smooth muscles is thus the combination of the two different mechanisms. The muscles which contract under the effect of adrenaline are overly susceptible to its effect upon the cellular membrane. On the other hand these muscles which dilatate under the action of adrenaline respond better to its effect upon the cellular metabolism.

Thus the variable response of the smooth muscles towards the compounds which were studied by us might be also the combination of the two effects of these compounds — first their effect upon cellular metabolism and second upon the reactivity of the cellular membrane. The explanation of the exact mechanism of these processes requires additional investigation both of metabolic and electrophysiological nature.

CONCLUSIONS

1. Local anesthetic agents used in this study (diethyl aminoethyl ester of acetylsalicyl acid, diethyl aminoethyl ester of methylcoumarylic acid, novocaine, xylocaine, larocaine, pantocaine and percaine) displayed variable effects upon the isolated smooth muscles of the intestinal tract.
2. The smooth muscles of the small intestine, taenia coli and of the uterus respectively may respond differently to the same local anesthetic agent.
3. The response of a particular muscle towards some local anesthetic agents does not allow to draw any general conclusions as to the effect of the compound upon the muscles of some other organs of the body.

REFERENCES

1. *Bülbring E.:* Pflügers Arch., 1961, 273, 1.
2. *Clegg P. C.:* J. Physiol., 1963, 169, 73.
3. *Domenjoz R.:* Schweiz. Med. Wschr., 1946, 76, 1282.
4. *Hazard R., Rernier-Cornee A. R.:* Arch. Int. Pharmacodyn. Ther., 1955, 104, 214.
5. *Jerzmanowska Z., Orchowicz Z.:* Acta Pol. Pharm., 1956, 13, 12.
6. *Kraatz Ch. P., Gruber Ch. M., Lisi A. G.:* J. Pharmacol., 1950, 98, 111.
7. *Szadowska A., Ciborska H.:* Dissert. Pharm., 1957, 9, 9.
8. *Szadowska A., Ciborska H.:* Acta Pol. Pharm., 1958, 15, 245.