The toxic effects of certain mutagenic substances on three species of acridids

W. Chapco and S. Fuzeau-Braesch

Department of Biology, University of Regina, Regina (Canada), and Laboratoire de Biologie de l'Insecte, Université de Paris-Sud, F-91405 Orsay (France), 3 May 1978

Summary. The toxicities of 4 alkylating agents (EMS, MMS, DES and MNU) were compared using 5th instar males of *Locusta migratoria cinerascens.* The substances in order of (decreasing) toxicity were: MMS, MNU, DES and EMS. Treatment of different species, strains, stages and sexes revealed that the toxicity of EMS is not invariant with respect to these factors. Acridids compared to most insects are extremely radiosensitive; this study shows that they are 'mutagen-sensitive' also.

Grasshoppers and locusts constitute one group of organisms whose genetics have largely been ignored (for reviews, see White[1] and Rowell[2]). In this article, the toxic effects on acridids of 4 chemical substances, considered mutagenic in other organisms[3], are described. These data will conceivably be useful in future attempts to induce mutations in these economically-important insects. Acridids, compared to most insects studied to date, are extremely radiosensitive[4,5], their LD50's being somewhat similar to those of mammals. This article examines whether they are 'mutagen-sensitive' as well.

The 4 substances, all monofunctional alkylating agents are: EMS (ethyl methanesulfonate), MMS (methyl methanesulfonate), DES (diethyl sulfate) and MNU (N-methyl-N-nitrosourea).

The relative toxicitie. of the 4 substances were appraised using a laboratory strain of 4-day-old 5th-instar males of *Locusta migratoria cinerascens* (Sardinia; *gregarius* forms[6]). The employment of this stage was based largely on its 'handleability' rather than on any knowledge of the sensitivities of the various germ cell stages (yet to be determined). 1 substance (EMS, see below) was used in a further study to examine possible strain, species, stage and sex differences. For these experiments, the material consisted of an albino strain derived from the above species and 2 species of grasshoppers; *Aiolopus thalassinus* (Algeria) and a non-diapause strain (Saskatoon, Canada) of *Melanoplus sanguinipes.* 5th-instar males and 4-day-old adult males were tested for all species and strains except in the case of *A. thalassinus* in which only larvae were used. The only females treated were 5th-instars of *M. sanguinipes.* All locusts received 15 µl of substance; the grasshoppers, because of their smaller size, received 5 µl. Injections were made abdominally. The concentrations administered were: 0.005, 0.01, 0.05, 0.1, 0.2, 0.4 and 0.8 M. All solutions were aqueous except for DES which was prepared in a 20% ethyl alcohol solution (in order to disperse globules which would otherwise have been formed). Controls were injected with comparable volumes of distilled water or 20% alcohol. In the case of the instars, mortality counts were made until the last surviving larvae had molted. Deaths among adults were recorded until there was no longer any unusual mortality. All animals were maintained at a 12-h photo- and thermal-period (24–38 °C; 22–38 °C for *Melanoplus*).

The number of animals treated and their mortality are presented in table 1. While most animals succumbed at 0.8 M, there were some clear differences among the dosage responses. To evaluate these, median lethal doses (MLD) were calculated using the method of Spearman and Körber (Finney[7]). Their estimates, along with their standard errors, are set out in the 2nd column of table 2. Comparing the MLD's, it can be seen that the substances in decreasing order of toxicity are MMS, MNU, DES and EMS. In general, the sequence accords fairly well with findings in yeast[8], *Drosophila*[9] and mice[10]. Apparently, methylating compounds are considered more toxic than ethylating ones[11] and this is certainly the case here. One explanation is that depurination reactions which may follow alkylation of purine bases in DNA and eventually lead to strand breakage are more rapid for methylated purines than for ethylated purines[11].

EMS is the least toxic, its use, therefore, allows for a wider range of dosages which could be applied in any future study, including 1 involving mutagenesis. In order to assess its possible differential effects with respect to the aforementioned factors, it was necessary to 'correct' for body weight and to allow for the fact that different volumes had been injected. The 3rd column (table 2) lists lethal doses expressed in mg of substance injected per g animal weight. Of the 3 species, *Aiolopus* is the most sensitive and *Locusta* the least. There is practically no difference between the sensitivities of the larval and adult stages in wild type *Locusta;* adults are slightly more sensitive than larvae in the albino strain; in *Melanoplus,* male larvae are more sensitive than adults. Since cellular turnover is presumably more rapid during the larval stages, one would have

Table 1. Mortality (number dead/number treated) of *Locusta* in response to 4 alkylating agents and of different species, strains, stages, and sexes in response to EMS

		Concentration (M) Control	0.005	0.01	0.05	0.1	0.2	0.4	0.8
Locusta-L	EMS	1/12	1/10	0/10	0/10	0/10	1/10	8/10	10/10
	MMS	-	0/10	0/10	9/10	10/10	10/10	10/10	10/10
	DES	0/10	1/10	0/10	3/10	5/10	4/10	7/10	7/10
	MNU	-	-	1/10	3/10	10/10	10/10	10/10	-
EMS									
Locusta-A		0/10	0/10	1/10	0/10	0/10	0/10	0/10	9/10
Albino-L		0/10	-	-	-	1/10	2/ 8	4/ 5	4/ 4
Albino-A		0/10	0/10	0/10	1/10	0/10	1/10	3/10	12/12
Aiolopus-L		0/10	0/10	0/10	1/10	10/11	9/10	11/11	-
Melanoplus-L		0/10	0/10	3/10	0/10	1/10	5/10	9/10	10/10
Melanoplus-L♀*		0/10	0/10	0/ 9	0/ 9	1/10	1/10	9/10	10/10
Melanoplus-A		0/10	0/10	0/10	0/10	0/10	1/10	7/10	10/10

L, 5th-instar larvae; A, adult. *All are male otherwise.

expected larvae to be more sensitive for all species. In *Melanoplus*, female instars are the least sensitive of all. The albino strain is less resistant than its wild type counterpart, especially at the adult stage. Whether these variations reflect, for instance, differential repair mechanisms is open to speculation. It is interesting to note, however, the existance of a variety of strains of *Drosophila* (treated as larvae) which display variable degrees of sensitivity to MMS; male-female differences are also noted but are found to be strain-dependent[12]. Comparing the acridids with 2 other organisms, it can be seen that their range of response overlaps that of the mouse, but is about 6–17 times more sensitive than *Drosophila*. These results are somewhat paralleled by MMS but the differences are not as great as for EMS. It would appear, therefore, that the radiosensitive acridid is also mutagen-sensitive, at least for EMS and possibly MMS.

Table 2. Median lethal doses of alkylating agents introduced by injection into a number of organisms including 3 species of acridids

	MLD (molarity) ± SE	mg/g
Locusta-L*		
EMS	0.2828 ± 0.0381 (15 µl)	1.1095
MMS	0.0251 ± 0.0029 (15 µl)	0.0837
DES	0.1518 ± 0.0441 (15 µl)	-
MNU	0.0501 ± 0.0096 (15 µl)	-
EMS		
Locusta-A*	0.5404 ± 0.0726 (15 µl)	1.1079
Albino-L	0.2549 ± 0.0490 (15 µl)	1.0001
Albino-A	0.3821 ± 0.0654 (15 µl)	0.7834
Aiolopus-L	0.0719 ± 0.0107 (5 µl)	0.3656
Melanoplus-L	0.1416 ± 0.0329 (5 µl)	0.4949
Melanoplus-L (♀)	0.2639 ± 0.0317 (5 µl)	0.8429
Melanoplus-A	0.3249 ± 0.0412 (5 µl)	0.6232
Drosophila-A[9]	-	6.9884
Mouse-A[10]	-	0.45–0.50
MMs		
Drosophila-A[9]	-	0.25
Mouse-A[13,14]	-	0.05–0.12

*L, 5th-instar larvae, A, adult.

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Cholinergic and adrenergic innervation of the penis artery of the bull: Transmitter concentrations and synaptic vesicles

E. Klinge, O. Eränkö and N.O. Sjöstrand[1]

Department of Pharmacology, University of Helsinki, SF-00170 Helsinki 17 (Finland), Department of Anatomy, University of Helsinki, SF-00170 Helsinki 17 (Finland), and Department of Physiology, Karolinska Institutet, S-10401 Stockholm 60 (Sweden), 16 May 1978

Summary. The penile artery of the bull contained significant amounts of acetylcholine, noradrenaline and dopamine, and its axon profiles contained either numerous small granular or agranular vesicles, as well as some large granular vesicles. In the dorsal metatarsal artery, only noradrenaline and dopamine were detectable, and the axon profiles observed contained numerous small granular vesicles. In the penile artery, the axons with small agranular vesicles, probably cholinergic axons, were in close contact with axons containing small granular vesicles. It is suggested that, in the penile artery of the bull, one of the functions of the cholinergic nerves is suppression of excitatory adrenergic neurotransmission.

In mammals, initiation of penile erection involves dilation of the penile artery as well as simultaneous relaxation of other smooth muscle effectors of erection, if present, i.e. of the retractor penis muscle and the muscles in the cavernous bodies[2]. It is likely that this functional entity of smooth muscles has an identical innervation consisting of an excitatory adrenergic component and 1 or more obscure inhibitory components. Thus in several mammals, neurogenic relaxation of these muscles is atropine-resistant and in vitro usual muscarinic concentrations of acetylcholine (ACh) do not relax but rather contract the muscles[2,3]. ACh is therefore hardly the inhibitory transmitter acting directly upon the smooth muscle cells. However, evidence has been presented indicating that cholinergic nerves are present in the smooth muscle effectors of erection, and that the function of these nerves might be suppression of excitatory adrenergic neurotransmission[3,4]. But the evidence for this concept has hitherto been essentially confined to studies on the retractor penis muscle.

In the present study we have made an attempt to clarify further the innervation of the penile artery of the bull by measuring its ACh, noradrenaline (NA) and dopamine (DA) contents, and by examining the ultrastructure of its axon profiles. For the sake of comparison, the dorsal metatarsal artery of the same bulls was studied in an identical way. In the bull, the penile and the dorsal metatarsal arteries have about the same outer diameter, but the former has a more elastic and soft consistence. Contrary to the penile artery, the dorsal metatarsal artery seems to be devoid of inhibitory nerves[3].

Materials and methods. Bulls weighing 250–500 kg were killed in the slaughter house and bled. Samples of the stem of the penile artery were obtained within 20–50 min after killing and were cut from the area lying between the branches serving the cavernous bodies[3]. ACh was determined biologically on the superfused frog rectus abdominis muscle[5]. The average amount of tissue used for extraction was 1.4 g. NA and DA were determined spectrophotofluo-