Biochemical Pharmacology, Vol. 23, pp. 1559–1568. Pergamon Press, 1974. Printed in Great Britain

EFFECTS OF OPIOID NARCOTIC DRUGS ON ENERGY RESERVES OF SKELETAL MUSCLE—II

FURTHER STUDIES OF THE GLYCOGENOLYTIC ACTION OF METHADONE

DESMOND R. H. GOURLEY*

Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Va. 22903, U.S.A.

(*Received* 11 *July* 1973; *accepted* 9 *November* 1973)

Abstract—The addition of 5 mM methadone to isolated intact extensor digitorum longus and soleus (EDL + SOL) muscles of the rat resulted in the rapid breakdown of creatine-P and ATP. AMP and ADP concentrations were also reduced by methadone, indicating degradation of the nucleotides beyond the level of AMP. Comparison of the concentrations of the various energy reserves at different times after the addition of methadone indicated that the narcotic drug first triggered the breakdown of creatine-P, which was followed in turn by the breakdown of ATP and glycogen. Although *levo*-methadone is more effective pharmacologically than *dextro*-methadone, the two isomers were equally effective in reducing the glycogen content of EDL + SOL muscles. The narcotic antagonist naloxone did not antagonize the glycogenolytic action of either methadone or morphine. 2-Diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) and the α-isomers of propoxyphene were approximately as effective as methadone in reducing muscle glycogen. It was concluded that the glycogenolytic action of the opioid narcotic drugs, SKF 525-A and propoxyphene is not related to their pharmacologic actions *in vivo* but probably results from a nonspecific effect of these compounds on the membranes of the muscle fibers.

OPIOID narcotic drugs have been found to diminish greatly the glycogen content of the diaphragm muscle or the intact extensor digitorum longus and soleus (EDL + SOL) muscles of the rat *in vitro*.[1] The most effective glycogenolytic narcotic drug of those tested was methadone, but the decreased glycogen levels of methadone-treated muscles could not be accounted for on the basis of the effects of methadone on the activities of the enzymes glycogen synthase or phosphorylase.[1] The loss of glycogen produced by methadone, therefore, appeared to be secondary to some other effect on the muscles, possibly on other energy stores. Accordingly, one objective of this work was to investigate the effect of methadone on the content of ATP and creatine-P of EDL + SOL muscles.

If the glycogenolytic effect *in vitro* were related to the pharmacologic actions of the narcotic drugs *in vivo*, it could be a valuable tool in studies of the mechanism of action of the opioid narcotic drugs in nerve tissue, particularly of the mechanism of drug tolerance and dependence. Certain pharmacologic actions of opioid narcotic drugs are reversed by narcotic antagonist drugs and typically are characteristic of the *levo* rotatory isomer of narcotic drugs that have two stereo isomers. Thus, a

* Present address: Department of Pharmacology, Eastern Virginia Medical School, 358 Mowbray Arch, Norfolk, Va. 23507, U.S.A.

second objective of this work was to investigate further the relationship between the glycogenolytic action of the narcotic drugs and their pharmacologic actions *in vivo* by utilizing the differentiating properties of a narcotic antagonist, naloxone, and of the two enantiomers of methadone.

MATERIALS AND METHODS

Preparation of isolated muscles. Male rats of the Sprague–Dawley strain purchased from Flow Research Animals, Inc. (Dublin, Va.) were allowed free access to food and water up to the time of use. The animals used in this series of experiments were part of a colony (N = 443) whose average individual weight was 74·3 ± 12·7 (S.D.) g at the time of decapitation. The EDL + SOL muscles were dissected from each hind limb of the rat and prepared for incubation in Krebs–Ringer solution (pH 7·4) containing 8 mM glucose exactly as described previously.[1]

Chemicals. All inorganic salts and glucose were analytical grade. The following drugs were generously supplied as gifts: 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) from Smith, Kline & French Laboratories; naloxone hydrochloride from Endo Laboratories; and α-*dextro*-propoxyphene hydrochloride, α-*levo*-propoxyphene hydrochloride, *dextro*-methadone hydrochloride, and *levo*-methadone hydrochloride from Eli Lilly & Co. Racemic methadone hydrochloride was purchased from Eli Lilly & Co. and morphine hydrochloride from Merck & Co.

Analytical methods. At the end of the incubation period, the muscles were blotted on filter paper and trimmed of their tendons. Muscles that were to be analyzed for their glycogen content were dropped into 30% potassium hydroxide to dissolve the protein in preparation for the isolation of glycogen by the method of Walaas and Walaas.[2] After hydrolysis of the glycogen, glucose was determined by the Glucostat (Worthington Biochemical Corp.) procedure.

Muscles that were to be analyzed for their adenine nucleotide and creatine-P content were frozen in liquid nitrogen. The muscles were sometimes stored at −70° for later analysis. The frozen muscles were powdered in a mortar chilled in liquid nitrogen. For the determinations of AMP, ADP and ATP, a weighed sample of the powder was stirred* with 5 vol. of 0·3 N perchloric acid for 3 min at 0° and centrifuged at 17,000 *g* for 10 min. The supernatant was adjusted to pH 7 with potassium hydroxide and the potassium perchlorate allowed to precipitate at 0° for 1 hr. The supernatant was removed after centrifugation at 1200 *g* for 15 min. AMP and ADP were determined by the enzymatic method of Adam[3] using myokinase, pyruvate kinase and lactic dehydrogenase. ATP was determined by the enzymatic method of Lamprecht and Trautschold[4] using hexokinase and glucose 6-phosphate dehydrogenase. After determining ATP, creatine-P was determined in the same sample by addition of ADP and creatine phosphokinase according to the method of Lamprecht and Stein.[5] Myokinase, pyruvate kinase, glucose 6-phosphate dehydrogenase and creatine phosphokinase were purchased from Boehringer Mannheim Corp. Lactic dehydrogenase and hexokinase were purchased from Sigma Chemical Co.

* In the experiment summarized in Figs. 1 and 2, the muscle powder was homogenized in perchloric acid with a Polytron PT 10 homogenizer for 15 sec at 0°. By this procedure, recovery of creatine-P was 100 per cent, but recovery of ATP was only 80 per cent. The ATP values in Fig. 1 are given without correction.

For the determination of adenosine 3′,5′-cyclic monophosphate (cAMP), the frozen muscle powder was extracted in 5% trichloroacetic acid with a Polytron PT 10 homogenizer. Further preparation of the sample and the determination of cAMP were carried out by the method of Gilman.[6] The protein content of the sample was determined by the method of Lowry *et al.*[7]

Statistical procedures. The significance of the difference between means was tested by conventional methods.[8] The paired variate procedure was used when the two means represented data from EDL + SOL muscles cross-paired from the same animals. The unpaired variate procedure was used when the two means represented data from muscles taken from different animals. (Values preceded by ± represent S.E.M. except where the S.D. is specified.)

RESULTS

Effect of methadone on muscle adenine nucleotides. In previous work,[1] extracts of EDL + SOL muscles which had been incubated without or with 5 mM methadone were passed through a column of Sephadex G-50 to separate the protein fraction containing the soluble enzymes from smaller molecules. The protein fraction was located by monitoring the effluent spectrophotometrically at 250 nm and it was closely followed off the column by a second fraction that also absorbed at the same wavelength. From this elution pattern and its absorption characteristic, the second fraction was assumed to contain nucleosides and nucleotides. It was observed regularly that the content of this fraction in extracts of methadone-treated muscles was only about one-fifth that in extracts of control muscles, whereas the content of the first fraction was about equal in both extracts. To determine whether the reduction in the second fraction of the extracts of methadone-treated muscles indicated a loss of adenine nucleotides, the effect of methadone on the content of AMP, ADP and ATP of muscles was investigated. One-half of the EDL + SOL muscles from three or four rats was pooled as one sample and several samples were incubated in the usual way for 1 hr. The cross-paired muscles of each sample were incubated in the presence of the same concentration of methadone as that used in the previous work (5 mM).[1] The results are summarized in Table 1. ATP virtually disappeared from the methadone-treated muscles, but it was not accounted for by the appearance of other adenine nucleotides. AMP and ADP were also markedly reduced in muscles exposed to methadone.

TABLE 1. EFFECT OF METHADONE ON ADENINE NUCLEOTIDE CONTENT OF EDL + SOL MUSCLES*

Nucleotide	Nucleotide content (μmoles/g) Control	Methadone	Methadone-treated (% of control)
AMP	0·051 ± 0·003	0·018 ± 0·001	35
ADP	0·293 ± 0·016	0·208 ± 0·011	71
ATP	1·38 ± 0·08	0·014 ± 0·003	1
AMP + ADP + ATP	1·73 ± 0·08	0·24 ± 0·01	14

* Each value is the mean ± S.E.M. of determinations on eight sets of pooled muscles. AMP, ADP and ATP were determined in each extract. The methadone concentration was 5 mM; the time of incubation was 1 hr.

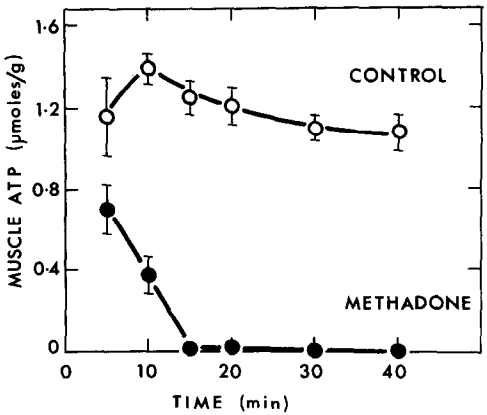

FIG. 1. ATP content of cross-paired EDL + SOL muscles incubated without or with 5 mM *dl*-methadone for various periods of time. Each point is the mean ATP content of six pairs of muscles. The vertical bars represent 2 S.E.M.

Adenosine was not detected in the extracts of either control or methadone-treated muscles. In a separate experiment in which muscles from two rats were pooled and incubated under similar conditions, the cAMP levels of cross-paired control and methadone-treated muscles were, respectively, 4·98 ± 0·37 and 3·33 ± 0·41 pmoles/mg of protein* (N = 11), but this represents a negligible contribution to the total amount of adenine nucleotides.

Time course of the effect of methadone on levels of ATP and creatine-P in muscles. To determine how rapidly methadone caused energy storage compounds to disappear from muscles *in vitro*, paired EDL + SOL muscles were incubated in the absence or presence of the same concentration of methadone for which similar time-course data for glycogen loss were available[1] (5 mM) for the various periods of time and then analyzed for their ATP and creatine-P content. The ATP content of muscles is shown as a function of time of incubation in Fig. 1. In the control muscles, the ATP level remained essentially unchanged for 40 min. In the presence of methadone, however, the muscle ATP was significantly reduced within 5 min and was practically all gone in 15 min. The creatine-P content of the same muscles is shown as a function of time of incubation in Fig. 2. In the control muscles, the creatine-P level increased during the first 30 min of incubation. In contrast, the creatine-P content of the methadone-treated muscles was already extremely low within 5 min and reached zero 15 min after addition of methadone.

Attempt to block the glycogenolytic action of methadone with naloxone. Naloxone is an extremely potent antagonist of several pharmacologic actions of the opioid narcotic drugs, including methadone.[9] If there is a relationship between the glycogenolytic action of methadone *in vitro* and its pharmacologic actions *in vivo*, naloxone would also be expected to antagonize, or block, the methadone-induced loss of glycogen. It was first necessary to determine that naloxone itself did not alter the glycogen content of EDL + SOL muscles. Data summarized in Table 2 show that con-

* One g wet weight of EDL + SOL muscles incubated under these conditions contained, on the average, 113 mg protein. This factor may be used to convert cAMP values to approximately the same units as the data in Table 1.

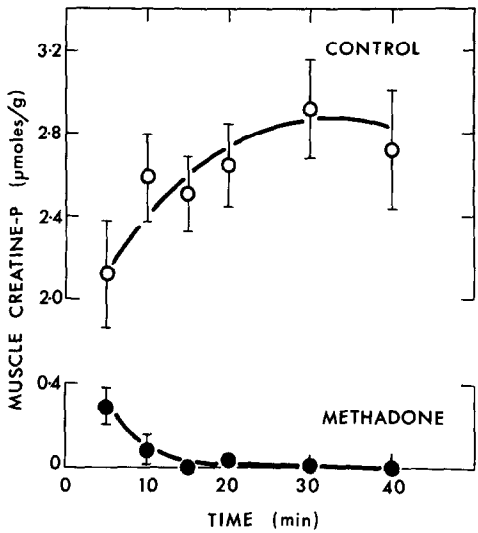

FIG. 2. Creatine-P content of the same pairs of muscles described in the legend to Fig. 1 (see Fig. 1 for details).

centrations of naloxone ranging from 0·2 to 5·0 mM had no significant effect on the glycogen content of the muscles under the usual experimental conditions.

In other experiments, methadone was added to both untreated and naloxone-pretreated muscles. The mean glycogen contents of both sets of muscles at the end of the incubation period are given in Table 3. When the concentration of methadone was 0·5 mM, there was no significant difference in the glycogen contents of the untreated and the naloxone-pretreated muscles, i.e. naloxone did not antagonize the glycogenolytic action of 0·5 mM methadone. When the concentration of methadone was 5 mM, the glycogen content of the naloxone-pretreated muscles was significantly lower than that of the untreated muscles. Thus, naloxone not only did not antagonize the glycogenolytic action of 5 mM methadone, but also added significantly to the glycogenolytic action.

A series of similar experiments was performed with morphine. Different concentrations of naloxone and morphine were tested, but antagonism of morphine's glycogenolytic action was never observed. One experiment, typical of the series, is summarized in Table 3. There was no significant difference in the glycogen content of the muscles treated with 5 mM morphine alone and the paired muscles treated

TABLE 2. EFFECT OF NALOXONE ON GLYCOGEN CONTENT OF EDL + SOL MUSCLES*

Concn (mM)	Glycogen content (μmoles/g) Control	Naloxone	P
0·2	15·1 ± 2·8	11·2 ± 1·5	>0·05
1·0	15·2 ± 2·6	12·6 ± 2·3	>0·1
5·0	12·8 ± 2·4	11·6 ± 2·9	>0·2

* Values given are the means ± S.E.M. of determinations on six pairs of muscles. The incubation period was 1 hr.

TABLE 3. EFFECT OF METHADONE OR MORPHINE ON GLYCOGEN CONTENT OF EDL + SOL MUSCLES PRETREATED WITH NALOXONE*

Drug	Concn (mM)	Glycogen content (μmoles/g) Untreated†	Naloxone-pretreated†	P
Methadone	0·5	18·8 ± 0·8	17·0 ± 1·4	>0·05
Methadone	5	11·4 ± 1·3	9·5 ± 1·0	<0·01
Morphine	5	10·2 ± 0·8	8·2 ± 0·5	>0·1

* Values given are the means ± S.E.M. of determinations on six pairs of muscles.

† In both methadone experiments, cross-paired muscles were incubated at 37° in 1·0 ml of either Krebs–Ringer medium or 5 mM naloxone. After 30 min, 0·5 ml methadone was added to both sets of muscles to give the final concentration stated in column 2. The methadone solution added to the naloxone-pretreated muscles contained 5 mM naloxone. Incubation continued for an additional 30 min. In the morphine experiment, the procedure was the same except that the naloxone concentration was 0·2 mM and the incubation period after the addition of morphine to both sets of muscles was increased to 60 min for a total incubation time of 90 min.

with 0·2 mM naloxone plus 5 mM morphine. Thus, antagonism of the glycogenolytic action of methadone or morphine by naloxone was not demonstrated.

Effect of d- *and* l-*methadone on muscle glycogen content.* Most of the pharmacologic activity *in vivo* of the racemic mixture of methadone resides in the *l*-isomer. In mice, for example, the analgesic potency of *l*-methadone is 30 times greater than that of *d*-methadone.[10] If the glycogenolytic action of methadone is related to its actions *in vivo*, *l*-methadone should be more potent than *d*-methadone when added to intact skeletal muscles *in vitro*. For this reason, the methadone isomers were tested at concentrations lower than those which produced the greatest effects when the racemic mixture was used. The data are summarized in Table 4. In the previous experiments, 0·1 mM *dl*-methadone reduced the glycogen content of EDL + SOL muscles in 1 hr to 84 per cent of the control levels.[1] In the present experiments, 0·1 mM of either the *d*- or the *l*-isomer did not significantly reduce the glycogen content of the muscles. As expected from this result, there was no significant difference in the glycogen contents of the muscles exposed to 0·1 mM *d*-methadone and those exposed to 0·1 mM *l*-methadone.

TABLE 4. EFFECT OF *dextro*- AND *levo*-METHADONE ON GLYCOGEN CONTENT OF EDL + SOL MUSCLES*

Isomer	Concn (mM)	Glycogen content (μmoles/g) Control	Methadone	P
dextro	0·1	7·00 ± 0·77	6·20 ± 0·53	>0·2
levo		8·83 ± 0·80 (P > 0·1)	8·14 ± 0·90 (P > 0·05)	>0·3
dextro	1·0	10·85 ± 0·75	4·23 ± 0·65	<0·001
levo		9·56 ± 0·84 (P > 0·2)	3·45 ± 0·25 (P > 0·2)	<0·001

* Values given are the means ± S.E.M. At 0·1 mM methadone, N = 9; at 1·0 mM methadone, N = 6.

In the second experiment of this series, the methadone concentration was 1·0 mM, which in the previous experiments with *dl*-methadone reduced the glycogen levels of EDL + SOL muscles to 48 per cent of the control levels.[1] In the present experiments, 1·0 mM of either *d*- or *l*-methadone significantly reduced the glycogen levels to, respectively, 39 and 36 per cent of the corresponding control levels. As expected from the similarity of these percentages, there was no significant difference in the glycogen contents of the muscles exposed to 1·0 mM *d*-methadone and those exposed to 1·0 mM *l*-methadone. Thus, a difference in the glycogenolytic action of the two enantiomers of methadone was not demonstrated.

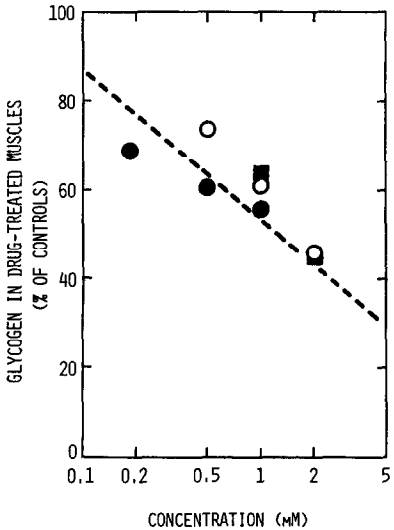

FIG. 3. Glycogen content of EDL + SOL muscles incubated for 1 hr with SKF 525-A (●), α-*d*-propoxyphene (○) or α-*l*-propoxyphene (■), expressed as a percentage of the glycogen content of the corresponding control muscles. All data shown represent muscles whose glycogen contents were significantly different from the corresponding control muscles at a probability level of 2 per cent or less. SKF 525-A at 0·1 M and α-*l*-propoxyphene at 0·5 mM were also tested but did not depress the glycogen content significantly. Each point in the figure represents the mean of six observations. The broken line represents the effect of *dl*-methadone on the glycogen content of EDL + SOL muscles taken from Fig. 1 of ref. 1.

Effect of SKF 525-A and α-d- *and* α-l-*propoxyphene on muscle glycogen content.* To test the hypothesis that the glycogenolytic property is related to chemical structure rather than pharmacologic activity, the effect on muscle glycogen of two drugs with chemical structures very similar to that of methadone, SKF 525-A and propoxyphene, were investigated, The results are shown in Fig. 3 in which the broken line indicates the relationship between glycogenolytic effect and methadone concentration described previously.[1] SKF 525-A was approximately as effective as methadone in reducing the muscle glycogen levels. The data for the glycogenolytic action of α-*d*- and α-*l*-propoxyphene indicate that: (1) there was no difference in the glycogenolytic effectiveness of the two isomers (although 0·5 mM α-*l*-propoxyphene did not have a statistically significant effect); and (2) propoxyphene, like SKF 525-A, was approximately as effective as methadone in reducing muscle glycogen content *in vitro*.

DISCUSSION

The time courses of the changes in the energy reserves of skeletal muscles exposed to methadone provide some insight into the mechanism of glycogenolysis induced by narcotic drugs. The changes in the ATP and creatine-P levels during the first 15 min after the addition of methadone derived from the data in Figs. 1 and 2 are compared in Table 5 with the changes in the glycogen levels in a different experiment derived from data in Fig. 2 of the previous paper.[1] Five min after the addition of methadone, the greatest decrease relative to the control values was observed in the

TABLE 5. CHANGE WITH TIME OF ENERGY RESERVES IN METHADONE-TREATED EDL + SOL MUSCLES COMPARED TO CONTROLS*

	Time (min)		
	5	10	15
Glycogen	82	53	39
ATP	61	27	1
Creatine-P	14	3	0

* Each value is the average content of six pairs of muscles treated with 5 mM methadone, expressed as a percent of the content of the paired control muscles. ATP and creatine-P were determined in the same muscles (Figs. 1 and 2); glycogen was determined in a separate experiment (Fig. 2 of ref. 1).

creatine-P levels. The next greatest decrease was found in the ATP content. Ten min after the addition of methadone, creatine-P had almost disappeared and the ATP content was very low. By 15 min, ATP also had almost disappeared from the methadone-treated muscles, but the glycogen content was still more than one-third of the corresponding control levels. Thus, creatine-P is the first of these energy reserves to be severely depleted by methadone. Next, the reserve of ATP is depleted. It appears likely that as the creatine-P levels fall, the ATP stores are utilized, and as the ATP levels fall, the glycogen stores are called upon.

Methadone undoubtedly penetrates the membranes of muscle fibers. Misra *et al.*[11] have reported that the partition coefficient (1-octanol–buffer, pH 7·4) of *l*-methadone was 55·5 ± 6·1 (S.D.)* It would be expected therefore that, very quickly after it was added to intact muscles *in vitro*, methadone reached the compartment in which creatine-P and ATP are stored and there triggered the breakdown of creatine-P in some way as yet unknown.

Several approaches to determine whether the glycogenolytic activity of opioid narcotic drugs *in vitro* is related to their pharmacologic activity *in vivo* have been described. The results of all of them are consistent with the conclusion that the glycogenolytic action in skeletal muscle is a nonspecific effect not related to the specific receptor–drug interaction which is thought to precede the pharmacologic actions of narcotic drugs *in vivo*. The glycogenolytic action was not antagonized by the pure narcotic antagonist naloxone, and *d*-methadone, which has only weak analgesic acti-

* The partition coefficient of morphine in the same solvent–buffer system was 1·02 ± 0·01 (S.D.),[11] which may partly account for the fact that morphine is only about 5 per cent as potent in its glycogenolytic effect as is methadone.[1]

vity, had the same glycogenolytic activity as the more potent analgesic isomer, *l*-methadone. The earlier observation[1] that the biochemical system which maintains glycogen levels was just as sensitive to methadone *in vitro* in muscles from rats which were tolerant to high daily doses of morphine as in muscles from naïve rats can probably be explained on this basis. Since the glycogenolytic effect is nonspecific, tolerance to it did not develop. The results of these experiments tend to exclude isolated skeletal muscle as a model system for studying the mechanisms of action of the opioid narcotic drugs in other tissues.

Further evidence that the glycogenolytic action of the opioid narcotic drugs is nonspecific derives from the observation that two drugs which are not opioid narcotics, SKF 525-A and propoxyphene, had essentially the same glycogenolytic potency as methadone. α-*Dextro*-propoxyphene, which has analgesic activity in mice,[12] had the same glycogenolytic potency as the nonanalgesic isomer, α-*l*-propoxyphene. The chemical structures of methadone, SKF 525-A and propoxyphene have several features in common. Meperidine, morphine and hydromorphone, which differ structurally from methadone in many respects, were found previously to have less glycogenolytic potency than methadone.[1] It seems likely, therefore, that the glycogenolytic effect of methadone is related to its chemical structure. As noted earlier, another factor which may play a role in the glycogenolytic effect of these drugs is their lipid solubility, which is a function of chemical structure.

The primary locus of the glycogenolytic action of methadone may be the cell membrane. Muscles incubated in the presence of methadone leak K^+ and degradation products of the adenine nucleotides.* Simon and Rosenberg[13] found that opioid narcotic drugs had a membrane effect similar to that of the local anesthetic drugs when applied to the squid axon. However, it is apparent that the squid axon membrane has no narcotic receptors such as those producing analgesia in other preparations because dextrorphan, the *dextro* form of levorphanol which is virtually devoid of analgesic activity, was about as effective as levorphanol in blocking axonal conduction. Moreover, ionic conductances in the squid axon are depressed equally well by both narcotic agonists and antagonists.[14] It is possible that the glycogenolytic action of methadone in skeletal muscle is similar to the nonspecific local anesthetic action of the opioid narcotic drugs.

Acknowledgements—This investigation was supported in part by NSF Grant GB 29558. The author is indebted to Kathleen N. Badal and Suzanne K. Beckner for technical assistance and to Dr. A. G. Gilman for kindly performing the analyses of muscle cAMP.

REFERENCES

1. D. R. H. Gourley, *Biochem. Pharmac.* **23,** 489 (1974).
2. O. Walaas and E. Walaas, *J. biol. Chem.* **187,** 769 (1950).
3. H. Adam, in *Methods of Enzymatic Analysis* (Ed. H.-U. Bergmeyer), p. 573. Academic Press, New York (1963).
4. W. Lamprecht and I. Trautschold, in *Methods of Enzymatic Analysis* (Ed. H.-U. Bergmeyer), p. 543. Academic Press, New York (1963).
5. W. Lamprecht and P. Stein, in *Methods of Enzymatic Analysis* (Ed. H.-U. Bergmeyer), p. 610. Academic Press, New York (1963).
6. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67,** 305 (1970).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
8. C. H. Goulden, *Methods of Statistical Analysis*, p. 40. Wiley, New York (1939).
9. H. Blumberg, H. B. Dayton, M. George and D. N. Rapaport, *Fedn Proc.* **20,** 311 (1961).

* D. R. H. Gourley, unpublished experiments.

10. D. G. LEIMBACH and N. B. EDDY, *J. Pharmac. exp. Ther.* **110,** 135 (1954).
11. A. L. MISRA, S. J. MULÉ, R. BLOCH and N. L. VADLAMANI, *J. Pharmac. exp. Ther.* **185,** 287 (1973).
12. N. B. EDDY, *Chemy. Ind.* 1462 (1959).
13. E. J. SIMON and P. ROSENBERG, *J. Neurochem.* **17,** 881 (1970).
14. D. T. FRAZIER, M. OHTA and T. NARAHASHI, *Proc. Soc. exp. Biol. Med.* **142,** 1209 (1973).