MOLECULAR PHARMACOLOGY, 23:344–349

cis-3,3′-Bis-[α-(trimethylammonium)methyl]azobenzene (*cis*-Bis-Q)

Purification and Properties at Acetylcholine Receptors of *Electrophorus* Electroplaques

JEANNE M. NERBONNE,[1] ROBERT E. SHERIDAN, LEE D. CHABALA,[2] AND HENRY A. LESTER[3]

Division of Biology 156-29, California Institute of Technology, Pasadena, California 91125

Received August 2, 1982; Accepted November 8, 1982

SUMMARY

The *cis* and *trans* isomers of the photoisomerizable compound, 3,3′-bis-[α-(trimethylammonium)methyl]azobenzene (Bis-Q), were purified by high-performance liquid chromatography using the ion-pair partitioning technique on a reverse-phase column. Solutions of *cis*-Bis-Q are stable at −20°; at 25°, thermal isomerization proceeds at a rate of 0.65%/day. *cis*-Bis-Q is less than 1% as potent a nicotinic agonist as the *trans* configuration. At concentrations of 1.5 μM or less, *cis*-Bis-Q exerts little or no blockade of the conductances induced by agonists. In voltage-clamped *Electrophorus* electroplaques exposed to *cis*-Bis-Q, laser flashes induce *cis*→*trans* photoisomerizations and increase the agonist-induced current by a factor of 20 within a few milliseconds.

INTRODUCTION

The *cis* and *trans* isomers of the photoisomerizable compound, Bis-Q,[4] differ markedly in their pharmacology at the nicotinic acetylcholine receptors of *Electrophorus electricus* (1, 2), several other fishes (3, 4), and cultured muscle (5). The *trans* configuration is a potent agonist; in most of the preparations mentioned, the concentration for half-maximal receptor activation is about 200 nM. On the other hand, little agonism is displayed by solutions containing predominantly the *cis* isomer. However, because samples of pure *cis*-Bis-Q have not been routinely available, it has usually not been possible to determine the magnitude of any contribution by the *cis* isomer to the measured agonist activity.

In addition to providing spectral data on the *cis* isomer, so that *cis/trans* isomer ratios could be measured accurately, a convenient preparation of *cis*-Bis-Q is of interest for two other reasons. First, this would allow more precise measurements on the pharmacological differences between the two isomers. Second, flash-induced photoisomerizations provide a way to produce "concentration-jumps" of *trans*-Bis-Q for kinetic and equilibrium measurements on the agonist-induced conductance (2, 6–9). If it can be verified that *cis*-Bis-Q has essentially zero agonist potency, such experiments could be started from effectively zero agonist concentration. This would avoid desensitization and would also eliminate conductance transients associated with *trans* → *cis* photoisomerizations of Bis-Q molecules already bound to receptors (6, 10).

Most synthetic routes lead to *trans*-Bis-Q. A chemical synthesis of *cis*-Bis-Q has also been accomplished,[5] and the product has been used in preliminary experiments (7, 11, 12). However, because the two isomers interconvert when exposed to light, it would be more convenient and less tedious to employ a chromatographic method for obtaining pure *cis*-Bis-Q from mixtures of the two isomers. HPLC has been combined with reverse-phase chromatography to enable the separation of a wide range of organic molecules, including structural isomers (13), *cis-trans* isomers (14, 15), and stereoisomers (16). Because reverse-phase chromatography involves aqueous solvents, it is particularly useful for many pharmacological agents.

Quaternary ammonium compounds, like other ionic compounds, pose a particular problem because they interact only weakly with some column packings and adhere too strongly for removal from others. An important advance in the separation and analysis of charged species was the development of ion-pair partition liquid chromatography, in which the inert support is coated with a hydrophilic liquid containing a counterion (17). In recent modifications of this technique, solutions containing the

This work was supported by National Institutes of Health Grant NS-11756.

[1] Recipient of a Fellowship from the American Heart Association.

[2] Recipient of a Fellowship from the Del E. Webb Foundation.

[3] Recipient of Research Career Development Award NS-272 from the National Institutes of Health.

[4] The abbreviations used are: Bis-Q, 3,3′-[α-trimethylammonium)methyl]azobenzene; HPLC, high-performance liquid chromatography.

[5] N. H. Wassermann, personal communication.

0026-895X/83/020344-06$02.00/0

counterion have been used as eluents in straight-phase and reverse-phase chromatography (18–20). This approach allows the separation of quaternary ammonium compounds in sharp, symmetrical peaks with reasonable retention times (21–24).

We have therefore exploited the ion-pair partitioning technique to separate the *cis* and *trans* isomers of Bis-Q and related disubstituted quaternary ammonium compounds by reverse-phase HPLC. A similar purification has been developed independently by Cash and Hess.[6] We have also characterized the action of *cis*-Bis-Q at *Electrophorus* electroplaques. As expected from previous work, the pure *cis* isomer shows very little agonist or antagonist activity at concentrations below 1.5 μM.

MATERIALS AND METHODS

Chromatography. HPLC analyses were performed on an Altex gradient liquid chromatography system coupled to a single-beam spectrophotometer (Hitachi, Model 100-10). The system employs a universal sample injector with a 2-ml sample loop and two single-piston pumps (Altex, Model 110A) fitted with pulse dampeners (maximal head pressure, 6000 psi) controlled by a digital programmer (Altex, Model 420). The stainless steel column is 15 cm long with a 4.6-mm inner diameter, packed with Ultrasphere-CN-bonded reverse-phase material (Altex, 10-μm particle size).

Small inorganic halides were expected to serve well as counterions because they form stable ion pairs with a variety of organic compounds. For analytical work (e.g., Fig. 1), perchlorate buffers were used. For preparative work, however, chloride was preferred, despite the risk of corrosion to the stainless-steel tubing of the HPLC system (17, 24), because this arrangement allows samples obtained from the HPLC to be diluted and used immediately in Ringer's solutions. Therefore, the mobile phases were as follows: Solvent A, 0.1 M NaCl in 0.1% HCl, pH 4; Solvent B, 60% CH_3CN and 0.1 M NaCl in 0.1% HCl. A linear elution gradient was employed, 0–50% Solvent B over a period of 60 min at 1 ml/min. To prepare the sample, an aqueous solution of *trans*-Bis-Q (10 mM, near the solubility limit) was irradiated with ultraviolet light with a peak energy at 366 nm (B-100 lamp, Ultra-Violet Products Inc., San Gabriel, Calif.) for at least 3 hr. Injection volumes were less than 100 μl for analytical work and 1–2 ml for preparative separations.

Analytical spectroscopy was performed on a dual-beam scanning spectrophotometer (Hitachi, Model 110).

Trans-Bis-Q was synthesized by Wassermann and Erlanger as described (7). Acetonitrile (HPLC-grade) was obtained from J. T. Baker Chemical Company (Phillipsburg, N. J.). Water was redistilled from $KMnO_4$ solutions. All other chemicals were Baker reagent-grade.

Electrophysiology. Agonist-induced currents were measured on isolated, voltage-clamped electroplaques from *Electrophorus electricus* (25, 26). Tetrodotoxin (10^{-7} M) and barium (3 mM) were added to the bathing solution in order to suppress noncholinergic, electrically excitable currents (25). In some experiments, the measurements were conducted while the preparation was exposed to flashes from a pulsed dye laser tuned to 440 nm (6, 10, 27).

RESULTS

Isolation and characterization of pure cis-Bis-Q. Under standard analytical conditions (perchlorate buffers), the *cis* isomer of Bis-Q elutes first in the gradient, at 18% acetonitrile and t_r = 30 min, and the *trans* isomer elutes at 25% acetonitrile and t_r = 37 min (Fig. 1). Preparative experiments (chloride buffers) were performed by increasing the injection volume to 1–2 ml. The order of elution was unchanged and, in fact, the *cis* isomer could be eluted in the void volume following large injections. In many experiments, the gradient was preceded by a 15- to 30-min elution with pure Solvent A to assure that the *cis* isomer was obtained free of CH_3CN. This result simplified the procedures because the *cis* samples, containing no acetonitrile, could be used directly in physiological experiments.

The optical absorption spectra (Fig. 2) are typical of azobenzene compounds and strongly resemble those previously published for Bis-Q (1, 6, 27). The spectra of the two isomers show an isosbestic point at 266 nm. At 320 nm, the absorption peak for the *trans* isomer, the absorption coefficients differ by about a factor of 20: the A_{320}/A_{266} ratio is 0.26 and 4.9 for the *cis* and *trans* isomers, respectively.

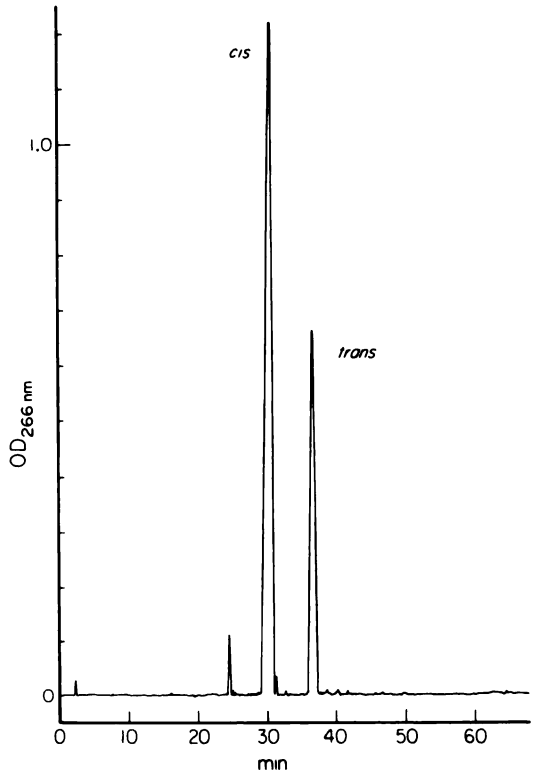

FIG. 1. *Elution profile of cis- and trans-Bis-Q obtained under standard analytical conditions: 0–100% Solvent B over a 60-min linear gradient at 1 ml/min*

Solvent A = 0.1 M $NaClO_4$ in 0.1% H_3PO_4; Solvent B = 60% CH_3CN and 0.1 M $NaClO_4$ in 0.1% H_3PO_4.

[6] D. Cash and G. Hess, personal communication.

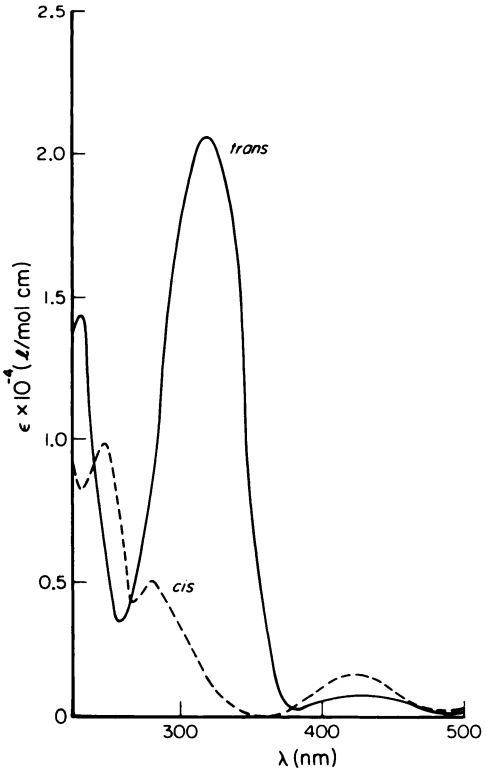

FIG. 2. *Optical absorption spectra of cis- and trans-Bis-Q, purified in a preparative run*

In mixtures of the two isomers containing no other absorbing species, the mole fraction of the *cis* isomer can be calculated by linear interpolation between these two values for A_{320}/A_{266} (cf. refs. 8 and 10). Using this procedure, we determined the thermal stability of the *cis* isomer (Fig. 3). Over a period of 30 days, there was no detectable thermal isomerization in solutions stored at −25° or at −67°; but at 25°, isomerization proceeds at a rate of about 0.65%/day, in agreement with the measurements of Duchek and Huebner (28). At Bis-Q concentrations below about 1 mM, the isomerization rate does not vary with concentration (Fig. 2). However, in preliminary experiments we found that isomerization proceeded at an accelerated rate in solutions of high ionic strength (more than ~1 M). We routinely store samples of Bis-Q collected from the HPLC at −25° or below for several months.

The 2,2′ analogue of Bis-Q also has properties of interest at nicotinic receptors (27, 29, 30), and we have used the technique described to separate the *cis* and *trans* isomers of this compound as well.

Pharmacology of cis-Bis-Q. Voltage-jump relaxations yield data on equilibrium and kinetic aspects of the response to agonists (31–33). In such experiments on *Electrophorus* electroplaques, *trans*-Bis-Q induces half-maximal conductance at a concentration of about 200 nM and a membrane potential of −150 mV (8). At the same concentration, however, *cis*-Bis-Q induced little or no detectable membrane conductance (Fig. 4). Pure *cis*- and pure *trans*-Bis-Q were converted, by exposure to visible light, to the same photostationary state, containing approximately 65% *trans* isomer. In experiments like those of Figs. 4 and 5, the two solutions then induced voltage-jump relaxations whose amplitudes and rate constants agreed to within 5% (data not shown).

In order to detect any pharmacological effects of *cis*-Bis-Q, experiments were conducted at higher concentrations. At 1.6 μM, *cis*-Bis-Q exerted no detectable blockade of the currents induced by agonists such as *trans*-Bis-Q (Fig. 5). We did consistently observe small agonist-induced currents in high concentrations (> 1 μM) of *cis*-Bis-Q (for instance, Episode 1 of Fig. 6). Such currents were not artifactual, because (a) they appeared both when the "divided pulse" procedure or straight subtraction was used to eliminate passive currents (25); (b) they were accompanied by depolarizations of 5–10 mV; and (c) they increased more than linearly with membrane hyperpolarization, as expected of agonist-induced currents. Currents of these amplitudes could have been induced by *trans*-Bis-Q concentrations of a few nanomolar, representing a contamination of less than 1%. This hypothesis is unlikely because the relaxation rate constants were several-fold faster than those produced by such a concentration of *trans*-Bis-Q (2, 6, 8) (although the relaxations were too small for accurate analysis). Therefore it appears that *cis*-Bis-Q is indeed a weak agonist and that it induces a channel with an average duration considerably shorter than that of *trans*-Bis-Q.

Direct values for the channel durations would come from patch-clamp recordings of single channels. It has been possible to obtain such records in several prepara-

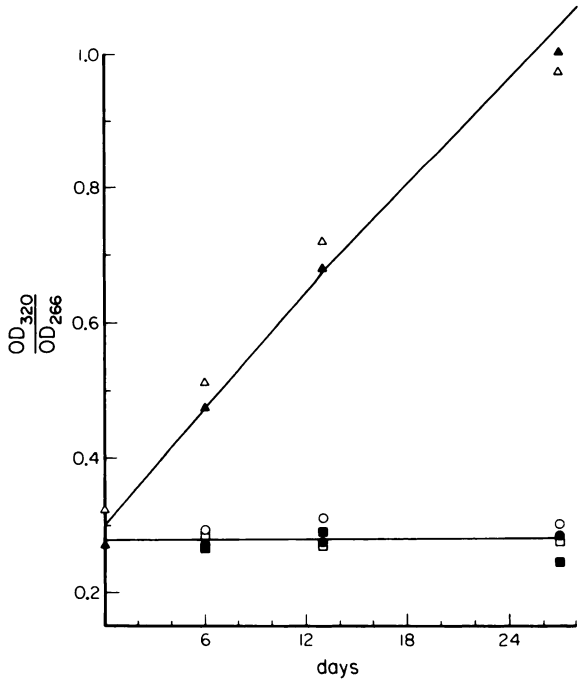

FIG. 3. *Thermal isomerization of cis-Bis-Q in 30 mM NaCl at 25° (△, ▲), −25° (□, ■), and −67° (○, ●)*

Kinetic experiments were performed at Bis-Q concentrations of 140 μM (*filled symbols*), 52 μM (*open symbols*), and 10 μM (data not shown), and all gave consistent findings. The line represents the beginning of an exponential approach to the pure *trans* isomer with a rate constant of 6.5×10^{-3} day^{-1}.

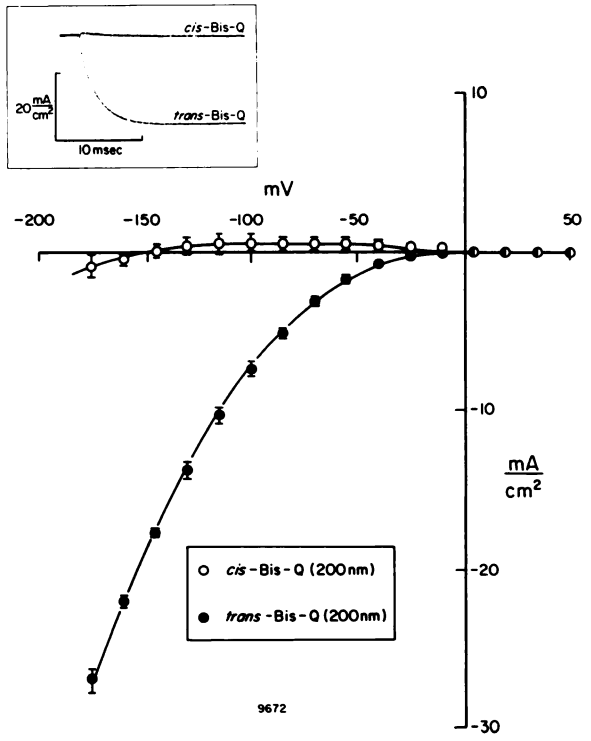

FIG. 4. *Currents induced by cis- and trans-Bis-Q (200 nM) in a voltage-jump relaxation experiment*

The voltage was jumped from +50 mV to the indicated potential, and currents were measured 20 msec after the jump. Passive currents have been subtracted. Data are means ± standard error of the mean. The *inset* shows actual traces of agonist-induced currents associated with the jumps from +50 mV to −175 mV. Temperature, 24°.

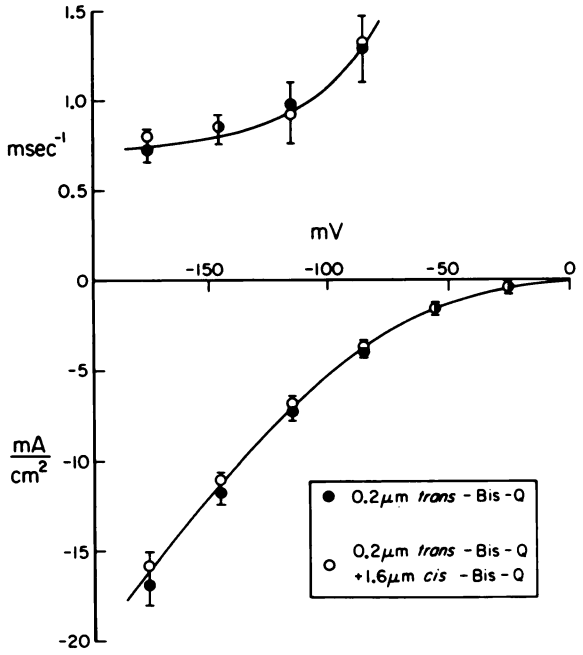

FIG. 5. *Effect of cis-Bis-Q (1.6 μM) on the response to trans-Bis-Q (200 nM)*

The *lower panel* shows agonist-induced current at the indicated voltage; the *upper panel* shows rate constants for voltage-jump relaxations to the indicated membrane voltage. ●, *trans*-Bis-Q alone; ○, *trans*-Bis-Q in the presence of *cis*-Bis-Q. Data are means ± standard error of the mean. Temperature, 24°.

tions with *trans*-Bis-Q but not with *cis*-Bis-Q (5); apparently, for the *cis* configuration, the channel opening rate is too low, the closing rate too fast, or both. One can estimate the ratio of lifetimes less directly by the following reasoning. In the experiment of Fig. 6, the large differences in current allow the conclusion that the voltage-jump relaxation in Episode 1 is dominated by the channel closing rate for *cis*-Bis-Q. As noted above, this relaxation cannot be measured accurately, but its rate constant is at least 3 $msec^{-1}$. On the other hand, the rate constant in Episode 3 (1 $msec^{-1}$) is dominated by the opening rate constant for *trans*-Bis-Q; because the *trans*-Bis-Q concentration is roughly 5 times the half-maximal value, the closing rate is roughly 0.2 $msec^{-1}$. Thus there is at least a 15-fold difference between the channel durations for the two isomers. Better temporal resolution is obtained in experiments where channels close soon after bound *trans*-Bis-Q molecules are photoisomerized to the *cis* configuration; such data suggest that the channel durations differ by at least 100-fold (6, 10).

We did not attempt to determine accurately the concentration of *cis*-Bis-Q required to induce half-maximal conductance, because apparent open-channel block is observed at higher concentrations. At 4 μM *cis*-Bis-Q, the agonist-induced conductance is smaller than that at 1.5 μM; i.e., the net effect was a slight blockade of current. At 4 μM, *cis*-Bis-Q also blocks currents induced by other, more potent, agonists—particularly at high negative potentials. For instance, in voltage-jump relaxation experiments with *trans*-Bis-Q (200 nM), relaxation rate constants were increased by about 20% and amplitudes were decreased by about 10% in the presence of 4 μM *cis*-Bis-Q at −175 mV. These effects typify the actions of "open-channel blockers" such as local anesthetics and suggest that, like many other compounds with quaternary ammonium groups and aromatic moieties, *cis*-Bis-Q can block open acetylcholine receptor channels at a concentration of several micromolar (34–37). Under the experimental conditions (responses near half-maximal conductance), a 25% blockade would imply that the *cis*-Bis-Q concentration equals the dissociation constant for binding to the open channel; the observed 10% blockade at 4 μM *cis*-Bis-Q implies a dissociation constant of about 18 μM at −175 mV (38).

Light-flash experiments were conducted on electroplaques exposed to *cis*-Bis-Q. In the experiment of Fig. 6, for instance, voltage-jump relaxations were recorded before and after a laser flash (in Episode 2) produced a concentration-jump of *trans*-Bis-Q. The light-flash relaxation itself produced more than a 20-fold increase in the agonist-induced current. This increase was stable on a time scale of milliseconds, but over the next second the preparation desensitized so that the agonist-induced current was decreased by 25% in Episode 3. As expected from previous work, the concentration-jump relaxation has a time course very similar to that of subsequent voltage-jump relaxations (2, 8).

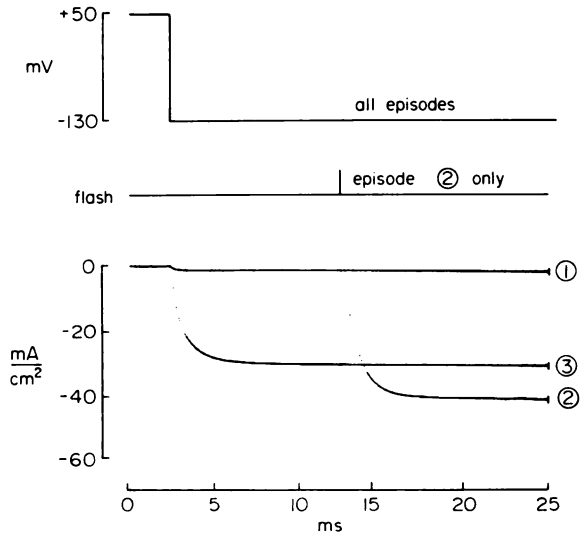

FIG. 6. *Voltage-jump and light-flash relaxations in a voltage-clamped electroplaque exposed to Bis-Q (1.5 μM)*

The trial comprises three voltage-clamp episodes, taken at intervals of 1 sec. *Lower traces* show agonist-induced currents; leakage and capacitative currents have been subtracted. At the start of the trial, the Bis-Q was in the pure *cis* form; 2.8 msec after the start of each episode, the voltage was jumped from +50 to −130 mV. For the first two episodes, there was only a small increase in current, showing that *cis*-Bis-Q is a very poor agonist. Thirteen milliseconds after the start of the second episode, a 440-nm laser flash occurred. The flash had a photoisomerization potency of 1.5 $flash^{-1}$, so that the *trans*-Bis-Q concentration was suddenly jumped to 757 nM (7, 10). As a result, the agonist-induced conductance increased exponentially to a much higher value; the rate constant was 1.07 $msec^{-1}$. The third voltage-jump relaxation occurred in the higher *trans*-Bis-Q concentration; the rate constant was 0.90 $msec^{-1}$. Temperature, 8°.

DISCUSSION

The photoisomerizable agonist Bis-Q has given useful information about the kinetics of channel gating at cholinergic receptors (reviewed in ref. 9), but certain experiments have until now been limited by the difficulties in obtaining and working with pure *cis*-Bis-Q. The present work shows that the compound can be obtained by a simple HPLC method and that it has the expected properties, including (a) reasonable thermal stability and (b) less than 1% the agonist potency of the *trans* isomer. Therefore, concentration-jump experiments can be started in the virtual absence of agonist. Such studies may yield further information about kinetic aspects of receptor activation. For instance, it might be possible to resolve a slower initial phase of the relaxation, corresponding to the time required for the binding of the agonist molecules. Other experiments may be directed toward the process of desensitization. For instance, single-channel measurements suggest that at least two kinetic components underlie this process in frog muscle (39). This suggestion leads to a prediction: after a concentration-jump of agonist, the agonist-induced current should decrease with more than one exponential component. These and related points are now being tested (4, 5).

As noted in the first investigations with Bis-Q (1), the wide discrepancy in the pharmacological properties of the two isomers is intriguing. The present data indicate that, although the *cis* isomer is not a competitive antagonist in the concentration range studied, it displays weak agonist and (at high negative voltages) channel-blocking activity at concentrations above 1 μM. This observation suggests that the *cis* isomer binds only weakly to the receptor (dissociation constant much greater than 1 μM). The *trans* isomer, on the other hand, binds very tightly, and previous findings indicate that (as seems to be the case for all other known agonists) the open state of the channel is much more likely to be associated with the presence of two bound *trans*-Bis-Q molecules than with a single molecule (10). Because the azobenzene moiety is conformationally rigid, Bis-Q and related molecules should give information about the nature of the binding site for agonists. It has been suggested that the receptor site has an electronic structure complementary to that of *trans*-Bis-Q (29). This hypothesis is amenable to investigation from the perspective of structure-activity relationships, because it is possible to alter the number and disposition of quaternary ammonium groups on the azobenzene backbone. It is interesting to note that the 2,2′analogue of Bis-Q displays antagonism (and no agonism) at *Electrophorus* electroplaques; furthermore, only the *cis* isomer has measurable potency (11, 30). Particularly because of the property of geometrical isomerization, we expect that this class of molecules will be useful in clarifying the electronic requirements for agonist, antagonist, and channel-blocking activity.

The chromatographic system employed here for the purification of *cis*-Bis-Q should be generally useful with other molecules of this type. We have already exploited this approach to purify the *cis* isomer of the 2,2′ analogue of Bis-Q.[7] Further investigations should be enhanced by the availability of both geometrical isomers in pure form.

ACKNOWLEDGMENTS

We thank Roger Spencer for assistance in all phases of the project, Mauri Krouse for performing some of the electrophysiological measurements, and N. H. Wassermann and B. F. Erlanger for furnishing Bis-Q.

REFERENCES

1. Bartels, E., N. H. Wassermann, and B. F. Erlanger. Photochromic activators of the acetylcholine receptor. *Proc. Natl. Acad. Sci. U. S. A.* **68**:1820–1823 (1971).
2. Lester, H. A., and H. W. Chang. Response of acetylcholine receptors to rapid, photochemically produced increases in agonist concentration. *Nature (Lond.)* **266**:373–374 (1977).
3. Krouse, M. E., H. A. Lester, and M. M. Weinstock. How photoisomerizable azobenzene compounds affect acetylcholine receptors of skate muscle. *Biol. Bull.* **157**:376 (1979).
4. Weinstock, M. M. Activation and desensitization of acetylcholine receptors in fish muscle with a photoisomerizable agonist. *J. Physiol. (Lond.)*, in press.
5. Chabala, L. D., H. A. Lester, and R. E. Sheridan. Single-channel currents from cholinergic receptors in cultured muscle. *Soc. Neurosci. Abstr.* **8**:498 (1982).
6. Nass, M. M., H. A. Lester, and M. E. Krouse. Response of acetylcholine receptors to photoisomerizations of bound agonist molecules. *Biophys. J.* **24**:135–160 (1978).
7. Krouse, M. E., M. N. Nass, J. M. Nerbonne, H. A. Lester, N. H. Wassermann, and B. F. Erlanger. Agonist-receptor interaction is only a small component in the synaptic delay of nicotinic transmission, in *Neurotransmitter and Hormone Receptors in Insects* (D. B. Sattelle, L. M. Hall, and J. G. Hildebrand, eds.). Elsevier/North-Holland, Amsterdam, 17–26 (1980).
8. Lester, H. A., M. E. Krouse, M. M. Nass, N. H. Wassermann, and B. F. Erlanger. A covalently bound photoisomerizable agonist: comparison with reversibly bound agonists at *Electrophorus* electroplaques. *J. Gen. Physiol.* **75**:207–232 (1980).

[7] J. M. Nerbonne, M. E. Krouse, R. W. Spencer, and H. A. Lester, unpublished observations.

9. Lester, H. A., and J. M. Nerbonne. Physiological and pharmacological manipulations with light flashes. *Annu. Rev. Biophys. Bioeng.* **11**:151–175 (1982).
10. Sheridan, R. E., and H. A. Lester. Functional stoichiometry at the nicotinic receptor: the photon cross-section for phase 1 corresponds to two Bis-Q molecules per channel. *J. Gen. Physiol.* **80**:499–515 (1982).
11. Lester, H. A., M. M. Nass, M. E. Krouse, N. H. Wassermann, and B. F. Erlanger. ACh receptor channels begin to open within 30 μsec after agonist is applied. *Soc. Neurosci. Abstr.* **4**:370 (1978).
12. Krouse, M. E., H. A. Lester, M. M. Nass, J. M. Nerbonne, N. H. Wassermann, and B. F. Erlanger. ACh receptors begin to open within 10 μsec after agonist is applied. *Soc. Neurosci. Abstr.* **5**:483 (1979).
13. Elnenaey, E. A., and W. P. Schoor. The separation of isomeric phenols of benzo(*a*)pyrene by high-performance liquid chromatography. *Anal. Biochem.* **111**:393–400 (1981).
14. Andersen, R. A., and T. R. Kemp. Reversed-phase high performance liquid chromatography of several plant cell division factors (cytokinins) and their *cis* and *trans* isomers. *J. Chromatogr.* **172**:509–512 (1979).
15. Smyth, M. R., and C. G. B. Frischkorn. Simultaneous determination of the *trans* and *cis* forms of zearalenone in cereal products by high performance liquid chromatography with voltammetric detection. *Anal. Chim. Acta* **115**:293–300 (1980).
16. Huggins, J. M., J. A. King, Jr., K. P. C. Vollhardt, and M. J. Winter. Separation of diasteromers, structural isomers, and homologs of n^5-cyclopentadienylcobalt and dinuclear molybdenum complexes by reverse phase high performance liquid chromatography using deoxygenated solvents. *J. Organomet. Chem.* **208**:73–80 (1981).
17. Schill, G., and E. Reid (eds.). *Assay of Drugs and Other Trace Elements in Biological Fluids.* Elsevier/North-Holland, Amsterdam, 87 (1976).
18. Knox, J. H., and J. Jurand. Separation of tricyclic psychosedative drugs by high-speed ion-pair partition and liquid-solid adsorption chromatography. *J. Chromatogr.* **103**:311–326 (1975).
19. Knox, J. H., and A. Pryde. Performance and selected applications of a new range of chemically bonded packing materials in high-performance liquid chromatography. *J. Chromatogr.* **112**:171–188 (1975).
20. Mellstrom, B., and R. Braithwaite. Ion-pair liquid chromatography of amitriptyline and metabolites in plasma. *J. Chromatogr.* **157**:379–385 (1978).
21. Muzzard, G., and J. B. Le Pecq. High-performance liquid chromatographic separation and trace determination of the antitumour derivatives of ellipticine and related quaternary ammonium compounds. *J. Chromatogr.* **169**:446–452 (1979).
22. Muto, G., and A. Nakae. Separation of the homologous series of alkylbenzyl dimethylammonium halides and alkylpyridinium halides by high performance liquid chromatography. *Chem. Lett.* 549–550 (1974).
23. Nakae, A., K. Kunihiro, and G. Muto. Separation of homologous alkylbensyldimethylammonium chlorides and alkylpyridinium halides by high performance liquid chromatography. *J. Chromatogr.* **134**:459–466 (1977).
24. Greving, J. E., H. Bouman, J. H. G. Jonkman, H. G. M. Westenberg, and R. A. DeZeeum. Analysis of quaternary ammonium compounds and basic drugs based on ion-pair adsorption high performance liquid chromatography. *J. Chromatogr.* **186**:686–690 (1979).
25. Sheridan, R. E., and H. A. Lester. Rates and equilibria at the acetylcholine receptor of *Electrophorus* electroplaques: a study of neurally evoked postsynaptic currents and of voltage-jump relaxations. *J. Gen. Physiol.* **70**:187–219 (1977).
26. Lester, H. A. Analysis of sodium and potassium redistribution during sustained permeability increases at the innervated face of *Electrophorus* electroplaques. *J. Gen. Physiol.* **72**:847–862 (1978).
27. Lester, H. A., M. M. Nass, M. E. Krouse, J. M. Nerbonne, N. H. Wassermann, and B. F. Erlanger. Electrophysiological experiments with photoisomerizable cholinergic compounds: review and progress report. *Ann. N. Y. Acad. Sci.* **346**: 475–490 (1980).
28. Duchek, J. R., and J. S. Huebner. Voltage transients from photo-isomerizing azo dye in bilayer membranes. *Biophys. J.* **27**:317–321 (1979).
29. Wassermann, H. N., E. Bartels, and B. F. Erlanger. Conformational properties of the acetylcholine receptor as revealed by studies with constrained depolarizing ligands. *Proc. Natl. Acad. Sci. U. S. A.* **76**: 256–259 (1979).
30. Krouse, M. E., H. A. Lester, B. F. Erlanger, and N. H. Wassermann. A study of the nicotinic acetylcholine receptor using a photoisomerizable competitive antagonist. *Soc. Neurosci. Abstr.* **8**:498 (1982).
31. Adams, P. R. An analysis of the dose-response curve at voltage-clamped frog endplates. *Pfluegers Arch.* **360**:145–153 (1975).
32. Neher, E., and B. Sakmann. Voltage-dependence of drug-induced conductance in frog neuromuscular junction. *Proc. Natl. Acad. Sci. U. S. A.* **72**:2140–2144 (1975).
33. Sheridan, R. E., and H. A. Lester. Relaxation measurements on the acetylcholine receptor. *Proc. Natl. Acad. Sci. U. S. A.* **72**:3496–3500 (1975).
34. Adams, P. R. Drug blockage of open endplate channels. *J. Physiol. (Lond.)* **260**:531–552 (1976).
35. Neher, E., and J. H. Steinbach. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol. (Lond.)* **277**:153–176 (1978).
36. Koblin, D. D., and H. A. Lester. Voltage-dependent and voltage-independent blockade of acetylcholine receptors by local anesthetics in *Electrophorus* electroplaques. *Mol. Pharmacol.* **15**:559–580 (1979).
37. Lester, H. A., M. E. Krouse, M. M. Nass, N. H. Wassermann, and B. F. Erlanger. Light-activated drug confirms a mechanism of ion channel blockade. *Nature (Lond.)* **280**:509–510 (1979).
38. Colquhoun, D., F. Dreyer, and R. E. Sheridan. The actions of tubocurarine at the frog neuromuscular junction. *J. Physiol. (Lond.)* **293**:247–284 (1979).
39. Sakmann, B., J. Patlak, and E. Neher. Single acetylcholine-activated channels show burst-kinetics in presence of desensitizing concentrations of agonist. *Nature (Lond.)* **286**:71–73 (1980).

Send reprint requests to: Dr. Henry A. Lester, Division of Biology 156-29, California Institute of Technology, Pasadena, Calif. 91125.